

**A Phase II Study of the Efficacy of Intravenous Umbilical Cord Blood Infusion  
as Cell Therapy for Children with Autism Spectrum Disorder (ASD):  
DukeACT**

**IND 15949**

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## INVESTIGATOR SIGNATURE PAGE

### **A Phase II Study of the Efficacy of Intravenous Umbilical Cord Blood Infusion as Cell Therapy for Children with Autism Spectrum Disorder (ASD): DukeACT**

I agree to conduct and supervise this clinical study in accordance with the design and specific provisions of this protocol; modifications to the study or protocol are acceptable only with a mutually agreed upon protocol amendment except when necessary to protect the safety of participants. I agree to await IRB approval for the protocol and informed consent before initiating the study, to obtain informed consent from participants prior to their enrollment in the study. I agree to report to responsible regulatory agencies and the IRB (when necessary) adverse events that occur in the course of this investigation. I agree to maintain accurate and adequate records in the case report forms as required by this protocol and maintain those records for the period of time required. I will make the study documentation available for safety oversight committee review and/or for other inspections as required. I agree to maintain study documentation for the period of time required. I agree to comply with all other requirements regarding the obligations of clinical investigators according to FDA regulations and guidance. I agree to ensure that all people assisting in the conduct of this study are informed in meeting the above commitments.

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(Investigator's printed name)

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(Investigator's signed name)

## PROTOCOL SUMMARY

Protocol Number:	DukeACT
Title:	A Phase II Study of the Efficacy of Intravenous Umbilical Cord Blood Infusion as Cell Therapy for Children with Autism Spectrum Disorder (ASD): DukeACT
Study Phase:	II
Study Site:	Single site; Duke University, Durham NC
Study Therapy, Dosage, and Route of Administration:	Autologous or allogeneic unrelated umbilical cord blood (CB), minimum pre-cryopreservation cell dose of $2.5 \times 10^7$ cells/kilogram, administered as a single intravenous infusion
Objectives:	<ol style="list-style-type: none"><li>1. To determine, in a randomized, placebo controlled, best available donor source trial, the efficacy of a single intravenous infusion of umbilical CB in improving the core symptoms of autism in young children with ASD.<ol style="list-style-type: none"><li>a. To determine whether there is a difference in mean response at 6 months between all cell sources and placebo.</li><li>b. To determine whether there is a difference in mean response at 6 months between autologous CB and placebo.</li><li>c. To determine whether there is a difference in mean response at 6 months between allogeneic CB and placebo.</li><li>d. To determine whether there is a difference in mean response at 6 months between autologous CB and allogeneic CB.</li></ol></li><li>2. To describe the safety and tolerability of a single intravenous infusion of unrelated donor CB in children with ASD.</li><li>3. To explore whether patient age or IQ correlate with response.</li><li>4. To explore whether changes in MRI, EEG, and eye tracking are observed after CB treatment.</li><li>5. To identify biomarkers that positively or negatively correlate with response to treatment.</li></ol>
Research Participant Population:	Up to 190 children ages two to seven years with ASD
Study Design:	This is a Phase II, single site, prospective, randomized, double-blind study of a single intravenous autologous or allogeneic, unrelated CB infusion. Subjects will be randomly assigned to <i>Sequence A</i> , consisting of a single infusion of CB cells at baseline followed 6 months later by a single infusion of placebo, or <i>Sequence B</i> , consisting of an infusion of placebo at baseline followed 6 months later by an infusion of CB cells. The primary endpoint will be evaluated 6 months after the first infusion.
Safety Assessments:	<ol style="list-style-type: none"><li>1. Incidence and severity of infusion reactions</li><li>2. Incidence and severity of product-related infections</li><li>3. Evidence of alloimmunization assessed by the presence of anti-HLA and anti-RBC antibodies and nonspecific markers of systemic inflammation (ESR, CRP)</li><li>4. Incidence and severity of any graft vs. host disease</li></ol>

5. Incidence and severity of adverse events, by relation to the study product

## ABBREVIATIONS

AE	Adverse Event
ASD	Autism Spectrum Disorder
CB	Umbilical Cord Blood
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CFU	Colony Forming Unit
CMV	Cytomegalovirus
CRF	Case Report Form
CNS	Central Nervous System
CP	Cerebral Palsy
CT	Computed Tomography
DMSO	Dimethyl Sulfoxide
DTI	Diffusion Tensor Imaging
FDA	Federal Drug Administration
GCP	Good Clinical Practice
CGI-I	Clinical Global Impression-Improvement
CGI-S	Clinical Global Impression-Severity
GMFM-66	Gross Motor Function Measure - 66
GvHD	Graft versus Host Disease
HIE	Hypoxic Ischemic Encephalopathy
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HSCT	Hematopoietic Stem Cell Transplantation
ICH	International Conference on Harmonisation
IRB	Internal Review Board
IV	Intravenous
MRI	Magnetic Resonance Imaging
MSCs	Mesenchymal Stromal Cells
PCR	Polymerase Chain Reaction
PDD-BI	Pervasive Developmental Disorder-Behavior Inventory
SAE	Serious Adverse Event
STCL	Stem Cell Laboratory
TNC	Total Nucleated Cells
VABS-3	Vineland Adaptive Behavior Scales, Third Edition



WBC

White Blood Cell

## 1.0 PURPOSE

The purpose of this study is to determine whether a single intravenous infusion of human umbilical cord blood (CB) can improve the core symptoms of autism in children with autistic spectrum disorder (ASD) through a randomized, placebo controlled, best available donor source phase II clinical trial.

## 2.0 BACKGROUND AND HYPOTHESIS

### 2.1 Autism Spectrum Disorder

Autism Spectrum Disorder (ASD) is a heterogeneous neurodevelopmental disorder with onset early in life. It is characterized by repetitive behaviors, a restricted range of activities, and impairments in social communication.<sup>1</sup> This disorder is 4 to 5 times more prevalent in boys and affects over 2 million individuals in the US, with approximately 1 in 68 American children identified as falling on the autism spectrum.<sup>2</sup> ASD is often accompanied by intellectual disability and is usually a chronic, disabling disorder that compromises the full potential of the affected individual. The majority of individuals with ASD are not able to live independently and require lifelong support or accommodations. Accordingly, the lifetime cost of supporting an individual with ASD is estimated to be \$1.4 million. The cost is \$2.4 million for those who also have an intellectual disability.<sup>3</sup>

The treatment of ASD is generally supportive and is often multimodal. Approaches include medication, behavioral therapy, occupational and speech therapies, and specialized educational and vocational support. All of the currently available medical treatments, such as psychotropic medications, are intended to ameliorate associated comorbid symptoms, such as irritability, but they are not disease-modifying. In light of this, there is a large unmet need for better, more effective and disease-modifying medical treatments for ASD.

The etiology of ASD is unknown, but recent studies indicate that genetic and environmental factors contribute to the disease. A common pathophysiological finding is abnormal synaptic functioning in areas of the brain, including the cerebral cortex. White matter abnormalities have also been reported, potentially implicating irregularities in myelination or axonal development in the pathophysiology of ASD.<sup>4</sup> In addition, abnormal functioning in aspects of the immune system in the brain (such as microglia that are tasked with providing support to neuronal synapses) has been described.<sup>5,6</sup> Increased plasma cytokine levels, upregulated genes associated with microglial activation, localized inflammation and pathological astrocyte activation have been associated with ASD.<sup>7</sup> **In this study, we hypothesize that infusion of CB will facilitate neural cell protection/repair and reduce inflammation resulting in improvement in the core autistic symptoms of children with ASD.**

Although the etiology of ASD is likely to be multifactorial, and the phenotypes can be variable, symptoms may be mediated by a final common pathway involving microglial activation. Nonetheless, it is possible that risk-based analyses performed at the conclusion of this study may uncover differential treatment responses in subsets of

subjects. Future studies utilizing induced pluripotent stem cells (IPSC) may be useful in further investigating any such findings.

## **2.2 Rationale for Cellular Therapy**

Human umbilical cord blood (CB) is rich in highly proliferative stem and progenitor cells mobilized by placental signals promoting homing to developing organs and is an established source of stem cells for hematopoietic transplantation. We have previously shown that allogeneic unrelated donor umbilical cord blood transplantation, after preparation with high dose, myeloablative chemotherapy, can halt progression of disease in children with inherited leukodystrophies.<sup>8-10</sup> In these patients, donor cells cross the blood brain barrier and engraft as microglial cells in the brain. We have also demonstrated the safety of intravenous autologous cord blood infusion in young children with brain injuries<sup>11</sup> and ASD. In several animal models of brain injury and cerebral palsy, it has been shown that administration of xenogeneic human CB leads to improved motor function.<sup>12</sup> Improvements in autistic symptoms have also been reported in two different mouse models of ASD after administration of bone marrow cells or mesenchymal stromal cells (MSCs). We hypothesize that cell therapy utilizing CB may improve the symptoms of ASD by modifying the immune response and inflammation in the brain and/or initiating repair of damaged brain circuitry.

## **2.3 Preclinical Studies of Cell Therapies in ASD**

Mouse models of ASD have been developed to provide a method to identify candidate genes and chromosomal regions associated with limited subpopulations of ASD such as Fragile X, Rett, and Angelman syndromes. Reported data from animal studies is confounded by the many differences in the phenotype of the ASD disorder.

Derecki, et al<sup>13</sup> report recovery of function in a model of Rett syndrome, an X-linked autism spectrum disorder usually caused by a mutation of the *MECP2* gene. This gene encodes a methyl-CpG-binding protein, and the mutation leads to deficient phagocytic function in glial cells. Transplantation of cells from wild type bone marrow via intravenous infusion arrested disease development in the mouse model of Rett syndrome (*Mecp2*-null C57BL/6 mice). Following engraftment, survival was improved, breathing patterns normalized, apneas were reduced, body weight increased, and locomotor activity was improved.

The BTBR *T<sup>+</sup> Itpr3<sup>fl</sup>* (BTBR) mouse strain, derived from the inbred Black and Tan BRachyury strain, is another mouse model of ASD. BTBR mice exhibit impaired social behavior, aberrant communication, increased repetitive behaviors, and increased cognitive rigidity. Segal-Gavish, et al<sup>14</sup> delivered human MSCs to BTBR mice via intraventricular injection into the central nervous system. Mice were immunosuppressed with cyclosporine before and after treatment. In this model, improvements in all three domains – social behavior, stereotyped behaviors, and cognitive rigidity – were observed in MSC-treated mice compared to controls. Differences in anxiety-related behaviors and locomotion were not observed.

These mouse models demonstrate the potential for benefit from cellular therapies in at least certain subtypes of ASD.

## 2.4 Preclinical Studies Demonstrating Mechanism of Action of CB Cells to Rescue/Repair Brain Cells

A common pathophysiological finding in ASD is abnormal synaptic functioning in the brain. Development and maintenance of neural synapses is a complex and continual process that begins early in development and continues throughout life. While synapses were once thought simply to be connections between neurons, it is now recognized that many different cell types, including astrocytes and microglia, play an integral role in synaptic pruning and maintenance. Microglia, in particular, play critical but incompletely understood roles in propagation and resolution of central nervous system injuries. These cells modulate neuroinflammation, produce factors that regulate activities of astrocytes, oligodendrocytes, and neurons, aid in maintenance of synapses, and clear debris to provide an environment for oligodendrocytes to begin to remyelinate neurons.<sup>15,16</sup> Preclinical data indicate that stem cell treatments have the ability to affect numerous cell types in the central nervous system via trophic or paracrine effects, thereby potentially altering the course of neurologic and neuropsychiatric diseases. Selected examples are detailed below.

### *Neurogenesis and Microglial Modulation:*

Microglia are thought to regulate changes in hippocampal neurogenesis through the production of inflammatory mediators such as tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin-1 beta and growth factors such as insulin-like growth factor-1 and brain-derived neurotrophic factor (BDNF).<sup>17-19</sup> Neuroinflammation inhibits neurogenesis in the adult hippocampus, and inflammatory blockade restores neurogenesis.<sup>20</sup>

Studies with human CB cells have shown benefit in xenograft models. In an aging rat model, human CB mononuclear cells have been shown to increase the proliferation of neural stem cells and decrease the number of activated microglia in the dentate gyrus of the hippocampus and the subgranular zone.<sup>21</sup>

More recently, Shahaduzzaman, et al<sup>22</sup> demonstrated that CB-derived T cells increase survival and proliferation of adult neural stem cells in vitro. In a mouse model, they showed that CB CD4<sup>+</sup> T cells promoted proliferation of neural stem cells in the dentate gyrus of aging rats and restored dendritic spine density of hippocampal pyramidal cells one week post-injection. Longer term, they demonstrated that a single IV injection of CB CD4<sup>+</sup> T cells increased proliferation in the dentate gyrus and decreased the amount of activated microglia in the aged rat brain. These results suggest that CB T cells may provide trophic support to aging neurons, thereby enhancing proliferation and maintaining dendritic and axonal connections in the aging brain.

### *Microglial Inflammation:*

In a mouse model of Alzheimer's disease, Lee et al. demonstrated that infusion of CB-MSCs resulted in reversal of disease-associated microglial neuroinflammation, as evidenced by decreased microglia-induced proinflammatory cytokines, elevated alternatively activated microglia, and increased anti-inflammatory cytokines. This was associated with reductions in amyloid- $\beta$  peptide deposition,  $\beta$ -secretase 1 levels, and tau hyperphosphorylation, known biomarkers of Alzheimer's disease. The mice also demonstrated improved spatial learning and memory decline. The authors suggest that

CB-MSCs produced their sustained neuroprotective effect by inducing a feed-forward loop involving alternative activation of microglial neuroinflammation, thereby ameliorating disease pathophysiology and reversing the cognitive decline associated with A $\beta$  deposition in Alzheimer's disease mice.<sup>23</sup>

*Oligodendrocytes & Myelination:*

Our group has developed DUOC-01, a cell therapy product cultured from banked human CB mononuclear cells.<sup>24</sup> Immunodepletion and selection studies demonstrate that DUOC-01 cells are derived from CB CD14<sup>+</sup> monocytes. In a NOD/SCID/IL2R $\gamma$ <sup>null</sup> mouse model of demyelination via cuprizone feeding, the corpus callosum of mice treated with DUOC-01 showed enhanced myelination (figure 1), a higher proportion of fully myelinated axons, decreased gliosis and cellular infiltration (figure 2), and more proliferating oligodendrocyte lineage cells than controls.<sup>25</sup> DUOC-01 cells also express higher levels of transcripts for several factors that can dampen inflammation, promote oligodendrocyte restoration, and promote myelination and tissue remodeling after injury. In addition, CD14 monocytes in thawed CB demonstrate anti-inflammatory and immunomodulatory activity, suggesting that without manipulation thawed CB cells have the capacity to exert anti-inflammatory and pro-neurogenic activity.



These and other preclinical studies indicate that CB cells may effectively modulate multiple types of cells in the central nervous system, including decreasing microglial activation and inflammation. Given the increasing evidence that synaptic and microglial dysfunction play a role in ASD, CB may be a candidate therapy for patients with ASD due to its ability to modulate microglial and other cells in the brain.

## **2.5 Treatment of Pediatric Patients with Neurological Conditions with *Autologous* CB**

At Duke, we have been studying *autologous* CB infusion for the past 11 years in over 600 children, ages one day to 15 years, with cerebral palsy (CP), hypoxic ischemic encephalopathy (HIE), hypoplastic left heart syndrome post ECMO, congenital hydrocephalus, ASD, and other brain injuries. No safety concerns were raised in this experience, which is detailed below.

### *Safety:*

The first trial, conducted from 2004-2009, confirmed the safety of administration of *autologous* CB as an intravenous infusion in the outpatient setting.<sup>11</sup> In this study, 184 infants and children with HIE, cerebral palsy, congenital hydrocephalus and other brain injuries received a total of 198 autologous CB infusions (14 patients received two infusions based on availability of larger cell doses). The majority of parents had elected to store their child's cord blood privately when they were born. Autologous CB units were obtained from 24 different cord blood banks: 149 (81%) CB units came from 11 private U.S. banks (113 from two of the larger private U.S. banks), 13 (7%) CBUs from 11 international banks, and 22 (12%) CB units from 2 public banks. The median volume of cord blood collected was 60 ml (range 5-180 ml) and median total nucleated cell count (TNCC) contained in the cord blood, as reported by the cord blood bank at the time of cryopreservation, was  $4.7 \times 10^8$  (range  $0.3$ - $33.8 \times 10^8$ ) total nucleated cells and  $1.8 \times 10^6$  (range  $0$ - $19.1 \times 10^6$ ) CD34 cells. All infusions were administered through a peripheral IV after premedication with oral Tylenol 15mg/kg, IV Benadryl 0.5mg/kg and IV Solumedrol 0.5mg/kg. Median post thaw recovery of TNCC was 82% (range 13-200%), and patients were dosed with a median of  $2.0 \times 10^7$  TNC/kg (range  $0.1$ - $13.3 \times 10^7$ ),  $0.7 \times 10^5$  CD34+ cells/kg (range  $0.04$ - $6.4 \times 10^5$ ), and  $6.5 \times 10^4$  CFU/kg (range  $0$ - $315 \times 10^4$ ).

Three patients (1.5%) experienced anaphylactic reactions during their CB infusion characterized by wheezing with or without urticaria 2-10 minutes after the IV infusion was initiated. The reactions resolved after discontinuation of the infusion and treatment with additional IV Benadryl and bronchodilators. The remainders of the CB cells were discarded for two of the infusions stopped prior to completion; one patient was able to restart and complete the CB infusion. One patient's mother experienced an allergic reaction consisting of urticaria, presumably due to contact with DMSO exhaled onto her face and neck by her child receiving a CB infusion. The reaction resolved with oral Benadryl. With up to 11 years of follow-up, no infections, autoimmune diseases, tumors, or other adverse events were observed.<sup>11,26-28</sup> This series demonstrated safety and feasibility of autologous CB infusion, and anecdotal reports of improved function were common.

### *Hypoxic Ischemic Encephalopathy (HIE):*

In phase I trial of newborns with HIE at birth conducted at Duke, fresh, non-cryopreserved *autologous* CB processed to reduce volume and RBC content on a Sepax 1 bioprocessor (Biosafe, Geneva) was infused in 1, 2, or 4 doses within the first 72 hours of life in babies with moderate-to-severe encephalopathy qualifying for therapeutic hypothermia.<sup>29</sup> These babies (n=39) were compared to a concomitant group of babies who were also cooled at Duke but did not receive CB cells (n=146). CB infusions were found to be safe in these critically ill babies, and babies receiving cells had increased survival rates to discharge (100% vs. 89%, p=0.03). Of the 25 CB recipients with known one-year outcomes, 16 (64%) survived with Bayley III scores  $\geq 85$  in all three domains, and of the 63 cooled-only infants with known one year outcomes, 25 (40%) survived with Bayley III scores  $\geq 85$  in all three domains (p = 0.04). A phase II randomized, placebo controlled, multicenter trial has recently been activated to formally test the efficacy of autologous CB infusion in these babies.

#### *Cerebral Palsy:*

The CP-AC study was a phase II, prospective, randomized, double blind, placebo controlled crossover study testing the efficacy of a single IV infusion of *autologous* CB in children ages 1-6 years with cerebral palsy (IND #14360). A total of 63 children with a median baseline age of 2 years (range 1-6) were enrolled and treated from 2010-2015 (32 CB, 31 placebo). Children were eligible if they were (1) Gross Motor Function Classification System (GMFCS) level 2-4 or (2) GMFCS level 1 with hemiplegia if they used their affected hand as an assist only. Children with known genetic conditions, intractable seizures, or severe microcephaly were ineligible. Autologous CB units were eligible if they had a precryopreservation TNCC of  $\geq 1 \times 10^7/\text{kg}$ , negative sterility tests, and negative maternal infectious disease tests. Subjects were evaluated at baseline, one year, and two years with functional evaluations (GMFM-66, Peabody, Assisting Hand Assessment, Bayley) and brain MRI with DTI tractography and connectivity analyses. They were randomized to the order of CB and placebo infusions (given one year apart). The placebo consisted of TC-199 tissue culture medium with 1% DMSO. The primary endpoint was change in GMFM-66 score at one year after the initial infusion (CB dosed at  $1-5 \times 10^7$  cells/kg or placebo). Cells or placebo were administered IV over 5-10 minutes in the outpatient setting after premedication with Tylenol, Benadryl, and Solumedrol. Subjects received IV fluids and were monitored for 2-4 hours post-infusion.

The median TNCC of CB infused in this study was  $2 \times 10^7/\text{kg}$  (range 0.8–4.8) and median infused CD34 dose  $0.7 \times 10^5/\text{kg}$  (range 0.1–4). Despite negative pre-cryopreservation cultures, one CB unit grew  $\beta$ -hemolytic strep upon thaw. There were no clinical infections, and no patients were treated with antibiotics. One participant had a transient infusion reaction with urticaria and fever, which resolved.

Analysis of the 63 patients at one year showed no difference in GMFM-66 change scores between placebo and treated groups (6.9 vs. 7.5, p=0.72). However, treated subjects with cell doses above or below the median precryopreservation or infused doses of  $3 \times 10^7/\text{kg}$  and  $1.98 \times 10^7/\text{kg}$ , respectively, demonstrated improvement in GMFM-66 change scores compared to subjects who received lower cell doses (p<0.01 for precryopreservation dose, p=0.05 for infused dose, Figure 2). Infused cell dose was not correlated with age (p=0.43) or type (p=0.32) or severity (p=0.46) of CP. Although the primary endpoint of

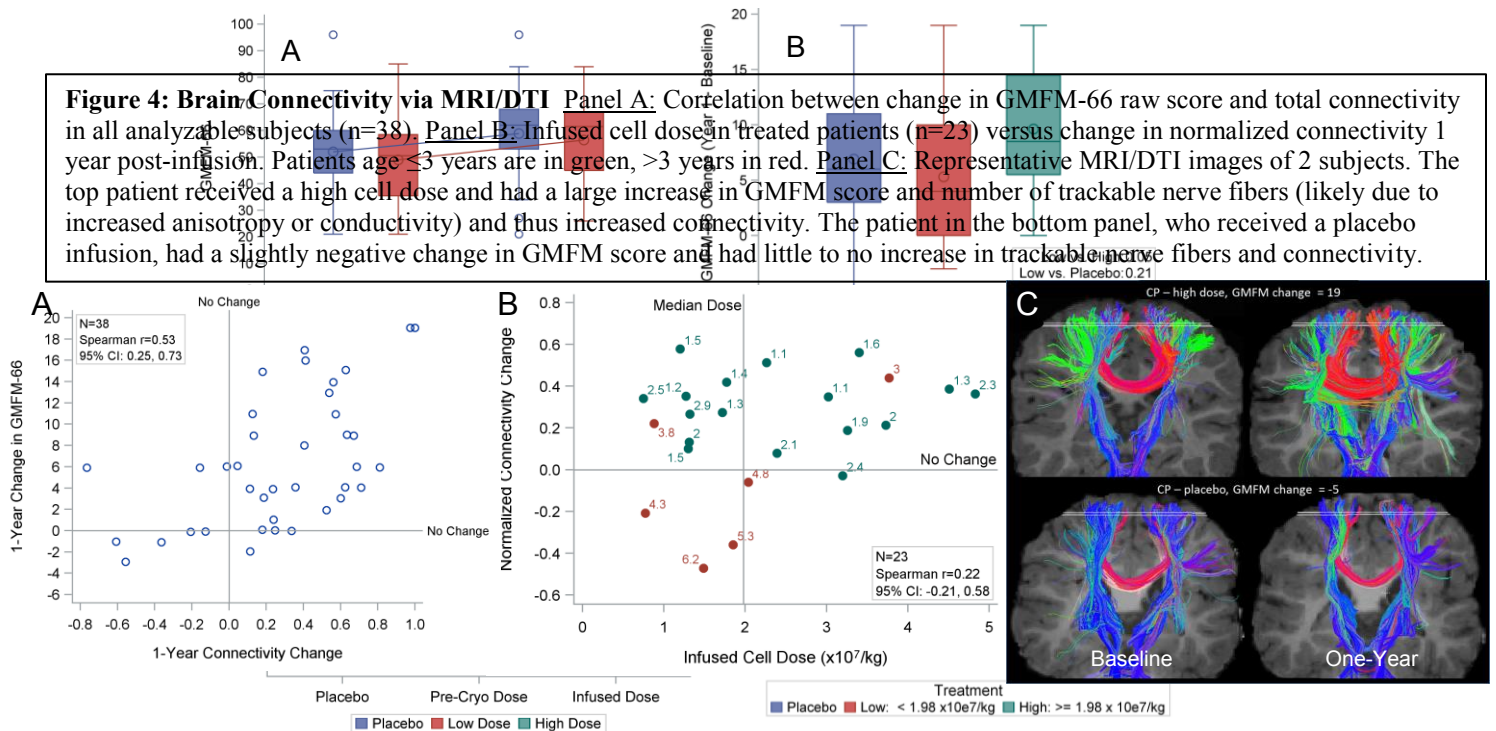


the study was change in the GMFM score in treated versus placebo patients 1 year post infusion, the observation that children receiving higher cell doses had a change in motor function, is important and influenced the minimum cell dose for this study (see section 6.4). The choice of this cell dose threshold is further supported by the fact that this same cell dose has been identified as the minimum therapeutic cell dose facilitating engraftment after allogeneic hematopoietic stem cell transplant.

**Figure 3: GMFM-66 Scores by Randomized Treatment Assignment and Cell Dose** Panel A: Distribution of GMFM-66 score at baseline and one year in patients randomized to placebo and autologous CB. Lines connect the group means (circles) over time. Panel B: Distribution of raw GMFM-66 change score from baseline to 1 year in subjects assigned to placebo and in treated subjects who received above or below the median infused cell dose (Low:  $<1.98 \times 10^7/\text{kg}$ , N=16 vs. High:  $\geq 1.98 \times 10^7/\text{kg}$ , N=16). Panel C: Raw GMFM-66 change scores based on median cell doses (Precryopreservation doses: Low,  $<3 \times 10^7/\text{kg}$ , N=16 vs. High,  $\geq 3 \times 10^7/\text{kg}$ , N=16; Infused doses:  $<1.98 \times 10^7/\text{kg}$ , N=16 vs. High:  $\geq 1.98 \times 10^7/\text{kg}$ , N=16). Panel D: Actual-Expected GMFM-66 scores in patients  $\geq 2$  years of age (N=38) at one year based on infused dose.

Using a subject's baseline GMFM-66 score, GMFCS level, age, and published percentiles,<sup>30</sup> we calculated the expected one-year GMFM-66 raw score for each patient. Since such percentile values are only available for children  $\geq 2$  years old, one-year old subjects (n=25) were excluded from this analysis. In the evaluable patients (n=38), the difference between the actual one-year GMFM-66 score and the expected one-year GMFM-66 score was calculated. In the entire autologous CB group, the median actual-expected difference in GMFM-66 scores was 1.7 (range -6.1-14.5), versus 2.2 (range -6.2-12.6) in the placebo group. When the CB group was analyzed by infused cell dose, subjects who received above the median infused dose of  $1.98 \times 10^7/\text{kg}$  (n=9) improved a median 4.3 (range -1.7 to 14.5) points greater than expected (p=0.05 vs placebo), whereas subjects who received below the median infused dose improved a median 1.9 points less than expected (range -6.1 to 12.9; p=0.02 vs high dose, p=0.07 vs placebo).

Forty-eight patients were also eligible for analysis of the Peabody Developmental Motor Scales-2 (PDMS-2), which assesses motor skills in children from birth through age 5. The median one-year change from baseline in the Gross Motor Quotient was 0.0 in the autologous CB group and -1.0 in the placebo group ( $p=0.38$ ). When the subjects treated with autologous CB were analyzed by infused cell dose ( $>/< 1.98 \times 10^7/\text{kg}$ ), a significant change was detected in the Gross Motor Quotient (3.0 vs. -2.0,  $p=0.02$ ).



MRI data was also analyzed to explore relationships between change in GMFM-66 scores, total brain connectivity, and cell dose. Accurate anatomical image parcellation could not be obtained in approximately one-third of subjects due to substantial morphologic brain abnormalities, leaving 23 treated patients and 15 placebo patients with usable connectivity data. However, the images from 2 of 4 patients  $\geq 3$  years who received high cell doses were unusable. There were no statistically significant differences in CP type, GMFCS level, or age between patients with and without analyzable images. As previously described,<sup>31</sup> there was a moderate correlation between change in GMFM-66 score and total connectivity at one-year in all analyzable subjects ( $n=38$ , Spearman  $r=0.53$ ; 95% CI: 0.25, 0.73;  $p<0.001$ ). Total connectivity change was not related to baseline GMFCS level, typography of CP, or gender, but was inversely correlated with age (Spearman  $r=-0.52$ ; 95% CI: -0.72, -0.23;  $p=0.001$ ). Though not statistically significant, there was a trend of increasing normalized connectivity at higher doses in the high-dose group, but no such relationship in the low-dose group.

The CP-AC was limited by small sample size of the dosing groups, as cell dose was not a pre-specified analysis. In addition to confirming safety of autologous CB infusion, the

CP-AC study demonstrated that when appropriately dosed, autologous CB infusion may be beneficial in children with CP.

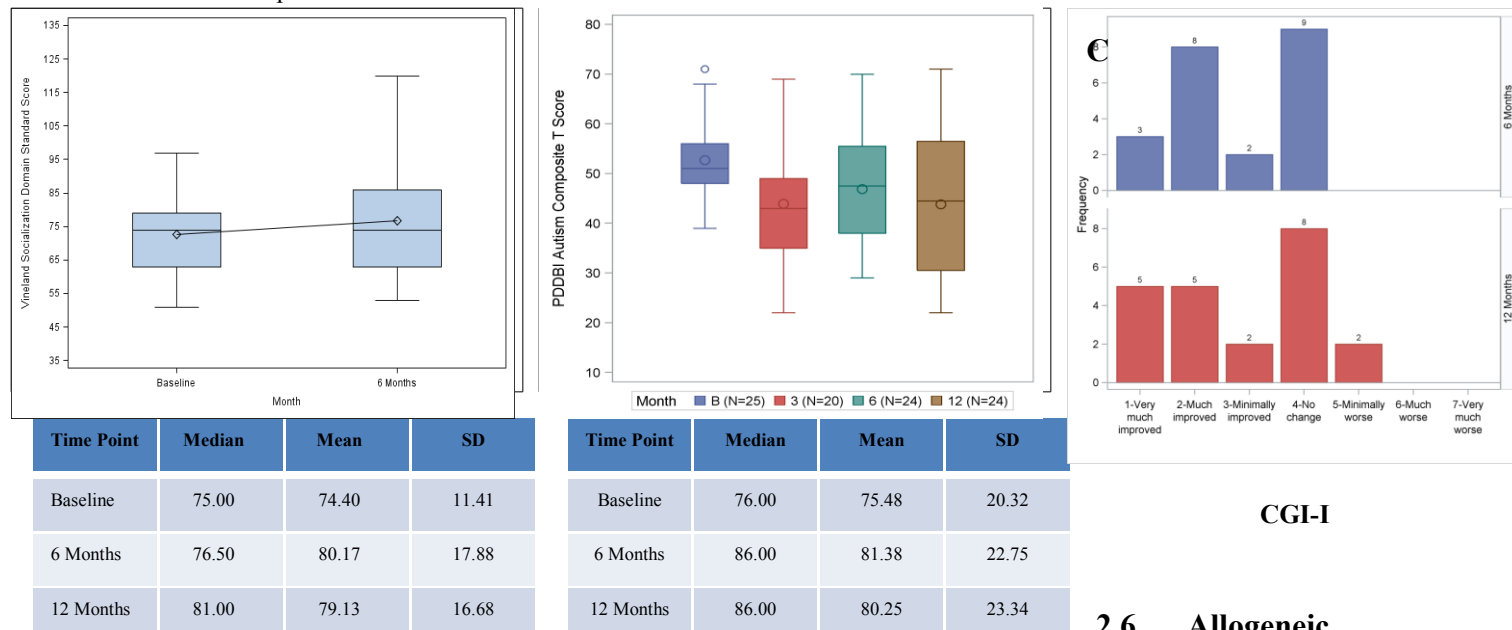
*Autism Spectrum Disorder (ASD):*

Duke investigators recently completed an open-label Phase 1 safety and tolerability study in 25 children (ages 2-5 mean age 4.5 years), diagnosed with ASD who were treated with a single intravenous infusion of **autologous** CB and followed for a year (ClinicalTrials.gov ID: NCT02176317). CB was administered as a single infusion (median infused dose:  $2.6 \times 10^7/\text{kg}$ , range:  $1.0 \times 10^7/\text{kg}$  to  $8.1 \times 10^7/\text{kg}$ ). No immunosuppression was implemented prior to infusion.

The safety and tolerability profile of autologous CB infusion in ASD was excellent. No serious adverse events were reported, and adverse events, in general, were sparse. Three children had mild allergic reactions associated temporally with the infusions, consisting of cough and hives during infusion for 1 child and cough post-infusion for 2 children. Also, one child was noted to be more irritable for 2 days post-infusion. No participants discontinued prematurely from the study due to adverse events.

With regard to preliminary assessments of efficacy, improvements in social communication abilities were noted on the caregiver-completed Vineland Adaptive Behavior Scales-Second Edition (VABS-II) and on the Pervasive Developmental Disorder-Behavior Inventory (PDD-BI) (figure 4). The Clinical Global Impression-Improvement scale, completed by clinicians, reflected beneficial changes during the 6-month period post-infusion in core ASD symptoms in approximately 60% of the participants, as manifested by the participants' increased social communication skills, receptive/expressive language, decreased repetitive behavior, and decreased sensory sensitivities. In computerized eye-tracking assessments, the participants manifested improvements in social attention, while their improvements in social behavior were found to be associated with increases in connectivity across brain regions when assessed via diffusion tensor imaging (obtained via MRI scans). This data suggests that a larger Phase 2 randomized study to evaluate the efficacy of CB therapy versus placebo is justified.

**Figure 5: Changes in ASD Symptoms after Autologous CB Infusion.** Panel A: Vineland-II socialization domain standard scores at baseline and six months after infusion ( $p=0.02$  baseline to six months). Panel B: Vineland-II communication domain standard scores at baseline and six months after infusion ( $p<0.01$  baseline to six months). Panel C: Distribution of CGI-I scores at six (blue) and 12 (red) months post-infusion. Sample sizes are  $N=25$  for baseline and 6-month time points and  $N=22$  at 12-months.



## Vineland Socialization Domain

## Vineland Communication Domain

## 2.6 Allogeneic Umbilical Cord Blood

Many children who might benefit from CB therapies will not have their autologous CB banked. In order to extend these therapies to all patients, use of an allogeneic product will be necessary. Allogeneic CB has been used extensively in the field of hematopoietic transplantation, and we have begun investigating its use in children and adults with brain injury. Background information is provided below.

### *Allogeneic CB for Hematopoietic Reconstitution:*

Allogeneic human CB as a source of cells for **hematopoietic reconstitution after myeloablative therapy** has a proven track record of over 25 years of use in the clinic, with over 35,000 transplants performed.<sup>32</sup> Allogeneic CB transplantation has been shown to be safe and has not been shown to cause tumors or cellular dysregulation. Compared to other cell sources, CB has the following advantages:

1. CB is an abundantly available source of stem cells that can be harvested at no risk to the mother or infant. It is routinely collected, cryopreserved, and banked.
2. Public CB donors are screened for risks of transmitting infectious agents through blood per CFR1271, subpart C donor screening regulations. Important infectious agents, particularly cytomegalovirus (CMV), are much less common in the newborn than adults, and are less likely to contaminate CB units.
3. CB units, cryopreserved and banked, are available on demand, and can be easily shipped and thawed for use when needed, eliminating delays and uncertainties that complicate bone marrow collection from unrelated donors.

4. CB lymphocytes are more immunotolerant of a new host. Thus, the intensity of graft-versus-host reactivity of fetal lymphocytes is less than that of adult cells, so transplantation of CB after myeloablation and immunosuppression where engraftment of donor cells is needed to treat the underlying disease results in less graft versus host disease (GvHD) than transplantation of bone marrow or other adult hematopoietic stem cell sources.
5. CB contains pluripotent stem cells that have demonstrated the ability to differentiate into numerous types of cells throughout the body, including in the brain. Thus CB may provide a source of cells for non-hematopoietic tissue repair or regeneration.
6. A minimum pre cryopreserved cell dose of 25 million cells per kg has been established as the threshold for successful hematopoietic engraftment after marrow and immune ablation.

In this study, we propose to use allogeneic CB donor without the use of chemotherapy or other immunosuppressive therapies. We are fully aware of the fact that engraftment of donor cells will not occur. Rather the mechanisms of action is paracrine signaling of endogenous cells to repair. Since there is no myelo or marrow ablation and only immunocompetent children will be treated, and engraftment is not expected, the risk of GVHD is virtually non-existent.

## 2.7 Preliminary Experience Using *Allogeneic* Cord Blood in Patients with Neurological Conditions

More recently, based on substantial preclinical data, CB has been investigated as a potential therapy for patients with brain injuries and neurological conditions. As all adults and most children do not have access to autologous CB, allogeneic cells are frequently utilized in clinical trials of cellular therapies for neurologic conditions. Examples of allogeneic CB studies in human patients are described below.

### *Stroke:*

The CoBIS study is an IRB approved, FDA IND sponsored, prospective, open-label, multi-center, Phase 1 safety study of a single intravenous infusion of non-HLA matched, ABO matched, ***allogeneic*** CB in 10 adults ages 18-80 years old. CB units are selected by ethnicity, blood type, and ability to supply a dose of  $0.5 - 1.5 \times 10^7$  TNCC/kg. Eligible patients include those experiencing a recent, acute cortical, hemispheric, ischemic stroke in the middle cerebral artery (MCA) distribution as detected by MRI as a diffusion weighted abnormality and are enrolled if their National Institutes of Health Stroke Scale (NIHSS) is 8-15 (right hemisphere) or 8-18 (left hemisphere). Participants who receive tPA or undergo mechanical perfusion are eligible for inclusion. Participants are not pre-treated with immunosuppressive drugs. The primary endpoint is safety as assessed by the frequency and severity of adverse events within 24 hours of CB infusion and during the 12 month period post CB infusion. Secondary outcome measures include Modified Rankin Scale (mRS), NIHSS, the Barthel Index (BI), and European Quality of Life (EQ-5D-3L), Patient Health Questionnaire Scale (PHQ8), Telephone Interview for Cognitive Status (TICS), and a self-reporting survey of rehabilitation therapy. MRI will be used to evaluate changes in the brain 3 months post infusion.

To date (March 2016), all 10 participants have been enrolled and treated with allogeneic unrelated donor non-HLA matched, ABO matched CB within 10 days of their stroke. There have been no serious adverse events with a follow-up of 1-8 months. Thus, early safety data suggests intravenous infusion of unmatched, **allogeneic**, CB cells is well tolerated. If the safety profile remains favorable, we will move to a randomized, placebo controlled Phase 2 study with the goal of using unrelated non-HLA matched CB to down-regulate inflammation and promote neuroprotection and neurorepair in patients with ischemic stroke.

#### *Cerebral Palsy at Duke:*

Given the benefit in motor improvement observed in the CP-AC study utilizing autologous CB, and recognizing that most children do not have a suitably qualified autologous CB unit available, we recently initiated a phase I clinical trial to evaluate the safety of fully HLA-matched or haploidentical **allogeneic** sibling CB infusion in children with CP (ClinicalTrials.gov ID NCT02599207). As of April 2016, 13 children have been treated. There have been no acute infusion-related toxicities, and no significant adverse events with short follow-up.

#### *Cerebral Palsy in Other Countries:*

Studies of allogeneic CB infusion in children with CP have also been conducted in Korea (n=105) and Russia (n=80). The Korean study had three groups: allogeneic CB + cyclosporine + erythropoietin; erythropoietin alone; and a control group.<sup>33</sup> One severely affected patient died in her sleep 14 weeks after CB administration, and this was determined to be unrelated to the CB infusion. Eight other patients experienced serious adverse events requiring hospitalization (pneumonia–4, seizure–1, influenza–2, urinary tract infection–1), but the distribution did not differ between groups. Non-serious adverse events that were more common in the CB group were pneumonia and irritability. At one year of follow-up, there were no reported prolonged or delayed serious adverse events. The authors reported greater improvements in cognitive and select motor functions in children who received CB and erythropoietin versus controls. There was no CB-only group for comparison. In the Russian study, children ages 1-12 years with cerebral palsy received 1-6 intravenous infusions of allogeneic CB with an average dose of  $2.5 \times 10^8$  viable cells per infusion.<sup>34</sup> Most patients who received four or more infusions showed improvement in tone, motor, and/or cognitive function, but there was no control group for comparison. In their series, factors that impacted treatment response included age, severity of brain damage, and number of CB infusions, with more responses observed in younger, less severely impaired children and those who received more than two infusions.

#### *Autism Spectrum Disorder (ASD) in Other Countries:*

In China, 37 autistic children, ages 3-12 years, were enrolled in a study utilizing allogeneic CB mononuclear cells (CBMNC) and/or umbilical cord-derived mesenchymal stem cells (UCMSC).<sup>35</sup> The children were divided into three groups: CBMNC (n=14), CBMNC+UCMSC (n=9), and control (n=14). All children received standard rehabilitation therapy. Cells were given in four doses, 5-7 days apart, via intravenous and/or intrathecal administration. In the cell recipients, transient fever (5/23) was the

only reported adverse event related to the therapy. Compared to control patients at six months, they observed greater improvements in the Childhood Autism Rating Scale (CARS) in the CBMNC+UCMSC group and in the Clinical Global Impression scale (CGI) in both cell groups. This was a small, non-randomized study designed primarily to assess safety. Nonetheless, the functional data suggests the potential for benefit.

## **2.8 Source of Unrelated Cord Blood Units for this Trial**

The Carolinas Cord Blood Bank (CCBB) is one of the largest public cord blood banks in the nation. Established in 1998 with support from the National Heart and Blood Institute of the NIH, the CCBB has over 30,000 CB units in inventory and has distributed over 2,500 CB units for transplant to date. In 2012 the CCBB received approval from the FDA for its BLA application to market DUCORD, a stem cell product derived from umbilical cord blood, for use in transplants between unrelated donors and recipients. DUCORD is approved for use in hematopoietic stem cell reconstitution for patients with disorders affecting the hematopoietic system that are inherited, acquired, or result from myeloablative treatment. The CCBB currently collects from 10 hospital sites (8 in North Carolina, 1 in Atlanta, GA and 1 in Boston, MA). It also accepts CB donations from mothers delivering in any hospital in North Carolina and Atlanta through a kit donation program.

## **2.9 Specifications for Qualification of Private or Public CB Units for Cell Therapy Studies**

Most hematopoietic stem cell transplants utilizing CB are carried out with allogeneic unrelated donor units obtained from public CB banks after myeloablative chemotherapy with or without total body irradiation. There are over 200 family CB banks worldwide that store autologous CB units after parental request and reimbursement. Most CB units are cryopreserved as red cell and plasma depleted, mononuclear cell enriched cell populations. Reference samples are also stored as attached segments or in vials for additional testing and qualification. High quality public and private banks screen maternal donors for the risk of transmitting blood-borne infectious diseases, as surrogates for the infant donor, with FDA-cleared donor screening tests in CLIA approved donor screening laboratories.

Based on established criteria utilizing allogeneic CB for hematopoietic stem cell transplantation and our experience in treating more than 600 children with autologous CB for neurological conditions, we have established the following criteria to qualify public or privately banked CB units for cell therapy studies. The CB unit must have:

1. Pre-cryopreservation total nucleated cell count (TNCC) documented and at least  $2.5 \times 10^7/\text{kg}$
2. Pre-cryopreservation viability  $\geq 70\%$  (total or CD34+ cell population)
3. Pre-cryopreservation sterility culture performed and negative
4. Maternal infectious disease screening as follows: Testing must include negative results for Hepatitis B, Hepatitis C, HIV, HTLV, and syphilis. These tests are superseded by the testing requirements of the country in which the cord blood was banked (ie. HTLV testing is not required or routinely performed in certain parts of the world where the infection is not endemic). Additional screening, which is

dependent on the timing of the CB collection, may be performed based on local and national regulations. If a screening test is positive, confirmatory testing must be negative, except for CMV. Units from mothers who have a positive CMV antibody screen may be used. Autologous units from mothers who test positive for Hep B core antibody but who test negative for Hep B Surface Antigen and Hep B NAT may be used.

5. Test sample available for identity confirmation and potency testing
6. HLA typing performed and meets study-specific parameters
7. CD45+ viability  $\geq 40\%$  or CD34+ viability  $\geq 70\%$  on thawed test sample

These same criteria will be utilized for this clinical trial and, along with procedures for CB administration, are detailed again in section 6.0.

## **2.10 Clinical Safety Summary**

In summary, in this study, we will administer a single intravenous infusion of either autologous or partially HLA-matched, allogeneic unrelated donor cord blood in young pediatric patients with autism. All children will have documentation of normal immune function prior to study enrollment. No chemotherapy or immunosuppressive pretreatment will be utilized. The infused cells are not expected to engraft. Rather they will exert a therapeutic effect via paracrine signaling which will stimulate endogenous cells to facilitate neural cell repair and connectivity.

Our experience in over 600 pediatric patients with brain injuries including Cerebral Palsy, HIE, ASD, and other injuries infused intravenously with autologous CB demonstrates that the procedure is safe and well-tolerated in children with neurological conditions and supports further testing in the pediatric ASD population. In addition, infusions in immunocompetent patients with brain injuries (adults with stroke and children with cerebral palsy), although smaller in number, also demonstrate a favorable safety profile and support further studies of allogeneic CB to treat children with ASD. Furthermore, infusions of allogeneic CB after myeloablative therapy in  $>35,000$  patients have been demonstrated to be safe for over 25 years, although in this setting because engraftment of donor cells must occur, a risk of graft versus host disease is present. This, however, is not the case in the current study because infused children are immunocompetent, will reject the donor cells and, accordingly, will not be at risk for GvHD.

## **2.11 Study Rationale and Hypotheses**

The major objective of this study is to determine whether a single intravenous infusion of CB can improve the core symptoms of autism in children with ASD. The rationale for the study and for the potential benefit of CB is based upon the following hypotheses:

- The developing brain exhibits remarkable plasticity, making young children ideal candidates for deriving maximal benefit from the pluripotential properties of CB.
- Cellular therapy, acting through paracrine or trophic mechanisms, may repair or facilitate improvement in areas of the brain with aberrant functioning by repairing or enhancing neural connectivity.
- Cellular therapy may modify immune response and inflammation that may be linked to improvements in the core symptomatology of ASD, including in areas such as social communication.



- Intravenous administration of allogeneic cord blood cells to immunocompetent hosts should be safe and effective. It is expected that the cells will be immunologically rejected within days to weeks of administration, eliminating a risk of Graft versus Host Disease (GvHD) or aberrant cell proliferation post-infusion.

The mechanistic rationale for this clinical study hypothesizes that CB can act through paracrine and allochrine mechanisms to modulate on-going inflammation and/or immune pathology in the brain and possibly protect neurons from further damage. In many contexts, CB cells dampen, rather than augment, immunological and inflammatory responses. Documented mechanisms include shifts in effector T cells such as generation of regulatory T cell populations and changes in monocyte/dendritic cell cytokine generation leading to anti-inflammatory cytokines. Therefore, it is plausible to consider a population of CB cells as an immunological and/or anti-inflammatory agent. Both postmortem brain tissue studies and PET imaging data from living individuals with ASD have revealed evidence of increased microglial activation, suggesting that immune and/or inflammatory mediated brain damage plays a role in the etiology of ASD as discussed above.<sup>36</sup> Thus, CB may be a candidate therapy for ASD because of the CB's immunomodulatory activities.

The projected rationale for this therapy focuses on the ability of CB to modify inflammation through downstream effects on glial cells and cytokines. CB cells may not be required to proliferate or persist in the recipient. Given that the recipients will not be conditioned in any way and have a full complement of stem and progenitor cells prior to treatment, we feel it is unlikely that allogeneic CB cells will engraft in the recipient or that engrafted stem and progenitor cells from the CB treatment will contribute significantly to any therapeutic effect.

## 2.12 Study Design

This is a single site, prospective, randomized, double-blind study of a single intravenous autologous or allogeneic, unrelated CB infusion in children ages 2-7 years with ASD. Participants will be randomly assigned to *Sequence A*, consisting of a single infusion of CB cells at baseline followed 6 months later by a single infusion of placebo, or *Sequence B*, consisting of an infusion of placebo at baseline followed 6 months later by an infusion of CB cells. All participants will ultimately be treated with CB cells at some point during the study. Participants with an available qualified autologous CB unit will receive autologous cells, and those without a suitable autologous CB unit available will receive cells from a  $\geq 4/6$  HLA-matched, allogeneic, unrelated donor CB unit from the Carolinas Cord Blood Bank. All infusions will be double-blinded. The primary outcomes will be assessed 6 months after the initial infusion in the sequence. Additional testing for secondary exploratory analyses will be performed at 12 months. Duration of study participation will be 12 months from the time of baseline infusion.

## 2.13 Risks and Benefits

CB cells will be prepared and infused using standard operating procedures that have been used in over 35,000 individuals worldwide. The potential risks associated with infusion of autologous CB cells or the placebo product include an allergic reaction to the product

(rash, shortness of breath, wheezing, difficulty breathing, hypotension, swelling around the mouth, throat or eyes, tachycardia, diaphoresis) to the product or transmission of infection. Additional risks associated with infusion of allogeneic CB cells include hypertension, bradycardia, anaphylaxis, hematuria, acute hemolytic reaction, rejection of cells, immune dysregulation (development of HLA directed antibodies), and development of graft-versus-host disease. All CB units are screened for infection and must meet release criteria prior to infusion, as described below. Participants will not receive immunosuppressive therapy prior to or after infusion of CB cells.

Participants will likely require sedation in order to complete a brain MRI and infusion. Risks associated with sedation include nausea, vomiting, blood vessel injury, nerve injury, lung injury, heart attack, allergy to medications, brain damage, respiratory insufficiency, hypoxia, hypotension, and anaphylaxis, and death. Medications used for sedation may include midazolam, dexmedetomidine, and/or propofol. Potential risks associated with the use of midazolam include excessive sleepiness or sedation, headache, hiccoughs, cardiac arrest, involuntary movements, agitation, changes in breathing (decreased, slowing or stopping of breathing), and decreased oxygen in the blood. Risks associated with the use of dexmedetomidine include increased or decreased blood pressure, nausea, vomiting, dry mouth, decreased red blood cells, fever, changes in heart rhythms (abnormal rhythms or a fast or slow heart rate), cardiac arrest, changes in oxygen levels or the amount of acid in the blood, fluid on the lungs, slowing of breathing. Risks associated with the use of propofol include pain at the injection site, nausea, vomiting, involuntary movements, changes in heart rhythm, heart failure, high blood pressure, inflammation of the pancreas (pancreatitis), anaphylaxis or severe allergic reaction, seizure, kidney failure, priapism (male erection that doesn't resolve without medical care), changes in breathing (including apnea or the stopping of breathing), increased acid in the blood, or infection in the blood. These associated risks are described in detail in the consent form. Risks associated with blood draws and IVs include momentary discomfort or pain, bruising, infection, bleeding, clotting, and fainting. Risks associated with genetic testing include medical, psychosocial, and economic risks, effects on insurability and employability, limits on educational options, and social stigma.

Potential benefits of this intervention include the possibility that the CB cells may, via direct or indirect mechanisms, induce changes that result in the reduction of the participant's core ASD symptomatology and improvement in abilities affected by ASD symptoms, such as social communication.

### **3.0 STUDY OBJECTIVES**

1. To determine, in a randomized, placebo controlled, best available donor source trial, the efficacy of a single intravenous infusion of umbilical CB in improving the core symptoms of children with ASD.
  - a. To determine whether there is a difference in mean response at six months between all cell sources and placebo.
  - b. To determine whether there is a difference in mean response at six months between autologous CB and placebo.

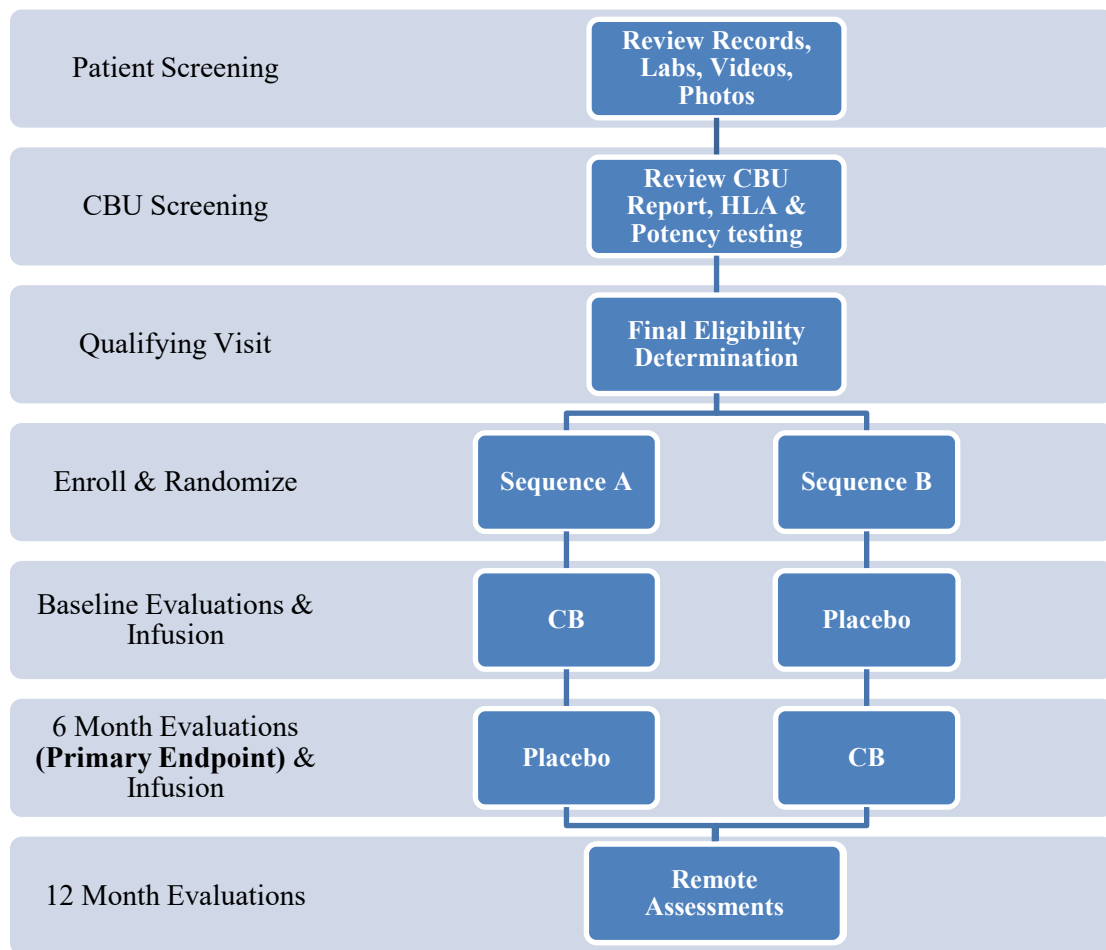
- c. To determine whether there is a difference in mean response at six months between allogeneic CB and placebo.
  - d. To determine whether there is a difference in mean response at six months between autologous CB and allogeneic CB.
- 2. To describe the safety and tolerability of a single intravenous infusion of unrelated donor CB in children with ASD.
- 3. To explore whether patient age or IQ correlate with response.
- 4. To explore whether changes in MRI, EEG, and eye tracking are observed after CB treatment.
- 5. To identify biomarkers that positively or negatively correlate with response to treatment.

## 4.0 STUDY DESIGN

### 4.1 General Design

This is a single site, prospective, randomized, double-blind study of a single intravenous autologous or allogeneic, unrelated CB infusion in children ages 2-7 years with ASD. Participants will be randomly assigned to *Sequence A*, consisting of a single infusion of CB cells at baseline followed 6 months later by a single infusion of placebo, or *Sequence B*, consisting of an infusion of placebo at baseline followed 6 months later by an infusion of CB cells. All participants will ultimately be treated with CB cells at some point during the study. Participants with an available qualified autologous CB unit will receive autologous cells, and those without a suitable autologous CB unit available will receive cells from a  $\geq 4/6$  HLA-matched allogeneic, unrelated donor CB unit from the Carolinas Cord Blood Bank. All infusions will be double-blinded. The primary outcomes will be assessed 6 months after the initial infusion in the sequence. Additional testing for secondary exploratory analyses will be performed at 12 months. Duration of study participation will be 12 months from the time of baseline infusion.

## 4.2 Study Flow Chart



## 4.3 Study Endpoints

### Primary Endpoint:

The primary endpoint of this study is the change in social communication skills (a core symptom of autism) from baseline to six months after the initial study infusion, as measured by the Vineland Adaptive Behavior Scale (VABS)-3 Survey Interview Form, Socializations Subscale Standard Score. Control (placebo) and treated patients will be compared.

### Secondary Endpoints:

Additional data regarding baseline to six month changes in autism symptom severity, problem behaviors, social, language skills, and treatment response will be assessed via both parent-reported and clinician-assessed measures, using the following measures:

1. Vineland Socialization domain raw score
2. Vineland Socialization domain age equivalent
3. Pervasive Developmental Disorder Behavior Inventory (PDD-BI) composite standard score (parent questionnaire)

4. CGI-S and CGI-I (clinician assessment)
5. Expressive One-Word Picture Vocabulary Test raw score (clinician assessment)
6. Vineland Adaptive Behavior Communication subscale standard score and age equivalent, Daily Living subscale standard score and age equivalent, and the Adaptive Behavior Composite standard score and age equivalent
7. Individual subscales of the PDD-BI t scores.

Safety and tolerability of donor CB infusion in children with ASD will be assessed by:

1. Incidence and severity of infusion reactions
2. Incidence and severity of product-related infections
3. Evidence of alloimmunization via anti-HLA and anti-RBC antibodies and nonspecific markers of systemic inflammation (ESR, CRP)
4. Incidence and severity of graft vs. host disease
5. Incidence and severity of unexpected adverse events, by relation to study product

Exploratory Analyses:

The following data will be collected for exploratory analyses:

1. Neurophysiological response to social and nonsocial stimuli via EEG and eye gaze tracking to social and nonsocial stimuli
2. Gastrointestinal symptoms via the Pediatric Quality of Life Inventory (PedsQL) Gastrointestinal Symptoms Scales
3. Brain connectivity via MRI with DTI

## **5.0 RESEARCH PARTICIPANT SELECTION AND WITHDRAWAL**

### **5.1 Study Population**

Up to 190 children ages 2-7 years with a confirmed diagnosis of ASD and an available qualified autologous or unrelated donor cord blood unit.

### **5.2 Inclusion Criteria**

1. Age  $\geq 2$  years to  $\leq 7$  years (7 years, 364 days) at the time of visit 1
2. Confirmed clinical DSM-5 diagnosis of Autism Spectrum Disorder using the DSM-5 Checklist
3. Fragile X testing performed and negative
4. Available and qualified umbilical cord blood unit with a minimum banked total nucleated cell dose of  $\geq 2.5 \times 10^7$  cells/kg that meets criteria outlined in Section 6.0, either:
  - a. Autologous umbilical cord blood unit OR
  - b.  $\geq 4/6$  HLA-matched allogeneic unrelated umbilical cord blood unit from the Carolinas Cord Blood Bank
5. Stable on current psychiatric medication regimen (dose and dosing schedule) for at least 2 months prior to infusion of study product
6. Normal absolute lymphocyte count ( $\geq 1500/\mu\text{L}$ )
7. Participant and parent/guardian are English speaking
8. Able to travel to Duke University two times (baseline and 6 months post-baseline), and parent/guardian is able to participate in interim surveys and interviews monthly
9. Parental consent

### 5.3 Exclusion Criteria

1. General:
  - a. Review of medical records indicates ASD diagnosis not likely
  - b. Known diagnosis of any of the following coexisting psychiatric conditions: depression, bipolar disorder, schizophrenia, obsessive compulsive disorder, Tourette syndrome
  - c. Screening data suggests that participant would not be able to comply with the requirements of the study procedures, including study outcome measures, as assessed by the study team
  - d. Family is unwilling or unable to commit to participation in all study-related assessments, including follow up for approximately 12 months
  - e. Sibling is enrolled in this (DukeACT) study
2. Genetic:
  - a. Records indicate that child has a known genetic syndrome such as (but not limited to) Fragile X syndrome, neurofibromatosis, Rett syndrome, tuberous sclerosis, PTEN mutation, cystic fibrosis, muscular dystrophy
  - b. Known pathogenic mutation or copy number variation (CNV) associated with ASD (e.g., 16p11.2, 15q13.2, 2q13.3)
3. Infectious:
  - a. Known active CNS infection
  - b. Evidence of uncontrolled infection based on records or clinical assessment
  - c. HIV positivity
4. Medical:
  - a. Known metabolic disorder
  - b. Known mitochondrial dysfunction
  - c. History of unstable epilepsy or uncontrolled seizure disorder, infantile spasms, Lennox Gastaut syndrome, Dravet syndrome, or other similar chronic seizure disorder
  - d. Active malignancy or prior malignancy that was treated with chemotherapy
  - e. History of a primary immunodeficiency disorder
  - f. History of autoimmune cytopenias (i.e., ITP, AIHA)
  - g. Coexisting medical condition that would place the child at increased risk for complications of sedation or other study procedures
  - h. Concurrent genetic or acquired disease or comorbidity(ies) that could require a future stem cell transplant
  - i. Significant sensory (e.g., blindness, deafness, uncorrected hearing impairment) or motor (e.g., cerebral palsy) impairment
  - j. Impaired renal or liver function as determined by serum creatinine >1.5mg/dL or total bilirubin >1.3mg/dL, except in patients with known Gilbert's disease
  - k. Significant hematologic abnormalities defined as: Hemoglobin <10.0 g/dL, WBC < 3,000 cells/mL, ALC <1000/uL, Platelets <150 x 10e9/uL
  - l. Evidence of clinically relevant physical dysmorphism indicative of a genetic syndrome as assessed by the PIs or other investigators, including a medical geneticist and psychiatrists trained in identifying dysmorphic features associated with neurodevelopmental conditions.

5. Current/Prior Therapy:
  - a. History of prior cell therapy
  - b. Current or prior use of IVIG or other anti-inflammatory medications with the exception of NSAIDs
  - c. Current or prior immunosuppressive therapy
    - i. No systemic steroid therapy that has lasted >2 weeks, and no systemic steroids within 3 months prior to enrollment. Topical and inhaled steroids are permitted.

#### **5.4 Research Participant Recruitment and Screening**

Patients may be recruited through IRB-approved advertising for the study on the websites of private CB banks, parent sponsored websites, the NMDP website, selected cerebral palsy societies, local medical providers, through a record of inquiries for previous studies (brain injury database), and through the Duke Center for Autism and Brain Development research registry. Separate IRB approval will be obtained for any advertisements.

Screening for the DukeACT study is conducted under a separate, IRB-approved screening protocol (Pro00063563). Under this protocol, after written informed consent is obtained from a parent/guardian, the patient's cord blood report (if applicable), medical records, school records, photographs, behavioral videos, and results of all genetic testing are obtained and reviewed by two teams. The medical review is conducted by a team of pediatric nurses, nurse practitioners, and physicians to identify the presence of any metabolic, immunologic, neurologic, sensory, genetic, or laboratory exclusion criteria. If no such exclusion criteria are identified, the psychiatric review is then conducted by a combination of psychologists and psychiatrists with expertise in diagnosing and treating children with ASD. They perform an extensive review of the patient's psychological records as well as any school and therapy records available. Both teams review the patient's photographs and records to evaluate for dysmorphic features. Any patients with questionable facial features or findings on genetic testing are then reviewed by a medical geneticist with expertise in genetic conditions associated with ASD. A patient must be approved by both medical and psychiatric screening teams to proceed with further laboratory or in-person screening and study enrollment. Should a concern for a previously undiagnosed condition or genetic finding arise during the screening process, this will be discussed with the patient's parent(s)/guardian(s) and a referral will be made to an appropriate medical or psychiatric provider for evaluation and treatment, if indicated.

#### **5.5 Early Withdrawal of Research Participants**

*Criteria for Removal from Protocol Therapy:*

1. Diagnosis of a genetic disease while under evaluation or on study.
2. Change in medical condition that precludes study participation.

Patients who are off protocol therapy are to be followed until they meet off-study criteria (see below). Follow-up data will be obtained on off-protocol participants unless consent is withdrawn. Participants that are taken off study prior to infusion of the CB will be considered not evaluable and can be replaced with another participant.

Off-Study Criteria:

1. Death.
2. Lost to follow-up.
3. Withdrawal of consent for any further data collection.
4. Completion of the final study visit.

## **6.0 STUDY PRODUCT**

### **6.1 Umbilical Cord Blood**

Patient enrollment is dependent on the availability of a banked unit of autologous CB at a private or public cord blood bank or an allogeneic unrelated donor CB from the Carolinas Cord Blood Bank. The unit must have appropriate degree of HLA matching, donor screening, and product characterization as detailed below.

### **6.2 HLA Matching**

All potential study participants will undergo HLA typing at HLA-A, B, and HLA-DRB1 via blood or buccal swab. HLA testing will be performed at low resolution for patients with autologous CB units and high resolution for patients utilizing allogeneic CB units. In addition to the HLA matching criteria, each CB unit must meet the infectious disease and dosing requirements detailed in the sections below. Matching requirements are as follows:

*Autologous CB units:* Identity confirmation via low resolution 6/6 HLA match.

*Allogeneic CB units:* Patients' HLA typing will be performed on two separate samples for confirmation. Allogeneic units that are potential matches will initially be identified from a search of the Carolinas Cord Blood Bank. The best available HLA-matched ( $\geq 4/6$ ) CB unit with a pre-cryopreservation nucleated cell dose  $\geq 2.5 \times 10^7$  cells/kg will be selected. Once a unit is selected, HLA typing will be used to confirm the original HLA typing and to select the best matching unit. When possible, at least 1 match at each HLA loci will be prioritized.

- A 6/6 matched donor will be selected before a 5/6, which will be selected before a 4/6 antigen matched donor. An UCB unit must be at least 4/6 HLA-matched with the patient.
- When a unit is partially mismatched, compatibility at the DR locus will be ranked as most important, followed by compatibility at the B locus and lastly the A locus.

### **6.3 Donor Screening for CB Units**

Screening of maternal blood collected within 30 days before or after delivery is used for CB donor infectious disease screening. Maternal testing must have been performed in a CLIA (or equivalent for CB units from international banks) certified donor testing laboratory. Testing must include negative results for: Hepatitis B, Hepatitis C, HIV, HTLV, and syphilis. These tests are superseded by the testing requirements of the country in which the cord blood was banked (ie. HTLV testing is not required or routinely performed in certain parts of the world where the infection is not endemic). Additional screening (i.e. West Nile, Chagas), which is dependent on the timing of the



cord blood collection, may be performed based on local and national regulations. If a screening test is positive, confirmatory testing must be negative, except for CMV. Units from mothers who have a positive CMV antibody screen may be used. Autologous units from mothers who test positive for Hep B core antibody but who test negative for Hep B Surface Antigen and Hep B NAT may be used.

#### **6.4 CB Unit Characterization and Shipping**

Results of initial testing at the cord blood bank must include a pre-cryopreservation TNC, viability and sterility culture. Pre-cryopreservation TNC must be  $\geq 2.5 \times 10^7/\text{kg}$ , sterility cultures must have been negative and total viability or CD34+ cell viability, if performed, must have been  $\geq 70\%$ .

A test vial or segment must be available from each CB unit for potency testing and confirmatory HLA typing for identity confirmation. The test sample will be sent/transferred to the Duke University Stem Cell Laboratory (STCL) in the frozen state. At the laboratory, the sample will be thawed and tested for viability, CFU, and CD34. HLA typing will be performed on the same or a related sample either at Duke or another certified laboratory. Units will be deemed acceptable for the trial if the CD45 cell population is  $\geq 40\%$  viable and/or the CD34+ population is  $\geq 70\%$  viable. CFU growth and CD34 will be described but will not be a specification for study enrollment.

After testing, CB units from CB banks outside of Duke will be shipped in a dry shipper containing a data logger to monitor temperature to the STCL at Duke University in the frozen state. Upon receipt, the CB unit will be examined for appropriate labeling and integrity of the cryopreservation bag and ports and the confirmation of the frozen state. The CB will be transferred from the dry shipper to a liquid nitrogen freezer and stored until the day of infusion.

On the infusion day, all or part of the CB will be thawed and washed with dextran 40 + 5% human serum albumin in the standard fashion per Standard Operating Procedure. At the time of thawing, standard studies including total nucleated cell count, viability, viable CD34+ cell count, CFU, and bacterial culture will be performed on the CB unit in the Duke STCL. A research sample of the CB will be retained for future analyses, and a sample of allogeneic CB units will be retained for later chimerism testing. A goal dose of  $2.5\text{--}5 \times 10^7$  TNCC/kg of participant body weight based on the pre-cryopreservation TNCC will be prepared for infusion in a volume of approximately 30 mL or no more than 5cc/kg as determined by the ordering physician.

#### **6.5 Placebo Product**

Cryopreserved UCB that has been thawed and washed in the standard fashion has a pink color due to the cellular and/or media content and a distinctive scent due to the DMSO used in cryopreservation. In order for patients and families to be truly blinded to the type of infusion they are receiving, the placebo product must be similar in both appearance and odor. Therefore, the placebo product will be acellular and will consist of TC-199 (pink) with 1% DMSO which are standard ingredients in cellular products. The volume of placebo product will be ~30 mL, in the range of a typical UCB unit that has been

washed and thawed after cryopreservation. The placebo solution will be placed in the same final container so the clinical staff remains blinded to study product or placebo.

#### **6.6 Packaging of Study Product**

All UCB products receive a unique identification number (ISBT Demand 128 bar code) to ensure integrity of the product and maintain chain of custody. The clinical site or cord blood bank will assign an ISBT Demand 128 bar code label to the CB unit, which is placed on the thawed CB unit bag or via tie tag. A similar label will be generated for the placebo products, which will be packaged in identical bags or syringes to ensure blinding of the patient and family. All products will be transported from the STCL to the infusion site in a validated cooler by courier.

#### **6.7 Administration of Study Product**

Patients will arrive in clinic on the morning of their scheduled infusion. A peripheral IV will be placed either by an anesthesiologist, clinical staff or study staff. After the CB is thawed and transported to the clinic, premedication with Benadryl 0.5mg/kg/dose IV, Solumedrol 0.5-1mg/kg IV will be administered. The infusion product (CB or placebo) will be administered intravenously over 2 to 25 minutes under direct physician supervision. Vital signs (heart rate, blood pressure, temperature, respiratory rate) will be checked upon arrival to the clinic and as clinically indicated. Pulse oximetry will be monitored continuously throughout the infusion and for 15-30 minutes post infusion. Patients will be observed and hydrated with standard IV hydration for approximately one hour post infusion. Patients will be discharged from clinic after a minimum of 1 hour providing all vital signs are at their baseline and they are awake, taking oral fluids, and asymptomatic with no evidence of toxicity.

#### **6.8 Safety Follow-up**

On Day 1 following the infusion, the participant will be seen by study staff to assess for any infusion related adverse reactions or complications. At ~2 weeks post administration of the CB, a member of the study team will contact the parent or guardian via phone or email to assess patient status and any adverse events. A questionnaire (see Appendix 1) will be administered at 3, 6, 9, and 12 months post-infusion to assess for serious adverse events. Included in the safety assessment, concomitant medications will also be collected at a minimum of at the baseline, 3, 6, 9, and 12-month time points.

### **7.0 STUDY PLAN**

#### **7.1 Overview**

Parents/Guardians who have previously contacted our program and have a child who may meet eligibility criteria for this study will be notified that this study is available. After initial contact, parents/guardians of potential research participants will have an initial phone interview with study personnel to describe the study, verify basic eligibility criteria, and confirm their interest in participation. The participant's eligibility will then be screened through review of medical records, video, photos, and laboratory testing under a separate screening protocol. Information on any autologous CB units will also be obtained and reviewed. If the child appears eligible, and does not have an autologous CB unit available, a suitable unrelated donor CB unit will be identified at the Carolinas Cord

Blood Bank. The CB unit (autologous or allogeneic) will be screened as described in section 6.

Once all screening is complete and the patient is likely to meet study criteria, the patient will travel to Duke for their first visit. On day 1, the informed consent will be obtained and patient eligibility will be determined by a physical observation and verification of ASD diagnosis per DMS-5 criteria. If the child is deemed eligible, they will be enrolled on study and randomized to the order in which they receive CB and placebo infusions (Sequence A or Sequence B). During their first visit, they will also have a brain MRI, neuropsychological evaluations, EEG, and their initial infusion (CB or placebo). Participants will be evaluated the day after the infusion and parents will be contacted 7-10 days after the infusion. Participants will return to Duke six months later, when they will undergo the same assessments and receive whichever infusion they did not receive at the first visit. Additional safety assessments will be performed via phone or email at 3, 9, and 12 months post-infusion.

## **7.2 Patient Screening**

Initial patient screening will be conducted with informed consent under a separate protocol and will include a review of medical records, videos, photos, initial laboratory testing, screening of autologous CB units as per section 5.4. If no exclusion criteria are identified, patient questionnaires and the Autism Diagnostic Interview- Revised (ADI-R), Shortened Version are administered. If a qualified autologous CB unit is not available, unrelated donor CB unit will be identified at the Carolinas Cord Blood Bank.

After study eligibility appears likely based on patient and CB criteria, potential participants will travel to Duke for initial evaluation. Evaluations and treatments will be conducted in the outpatient setting. After informed consent is obtained, a brief physical exam and baseline psychological testing will be conducted to confirm eligibility. If no exclusion criteria are realized, the patient will be enrolled, randomized, and undergo the remainder of the study evaluations.

## **7.3 CB Unit Selection**

If the child has an available, banked autologous CB unit, the CB report for that unit will be obtained from the bank at which it is stored, if not already available. If the autologous CB unit meets the donor screening and characterization requirements outlined in section 6, a sample of the patient's blood/buccal mucosa, and CB unit will be obtained for HLA typing confirm that the CB unit belongs to the child.

If a participant does not have a suitable autologous CB unit available, an allogeneic unrelated donor CB unit will be identified at the Carolinas Cord Blood Bank. HLA typing will be obtained on the patient, and the best available HLA-matched CB unit with a pre-cryopreservation nucleated cell dose  $\geq 2.5 \times 10^7$  cells/kg will be chosen according to the principles below.

- When possible, at least 1 match at each HLA loci will be prioritized.
- When a unit is partially mismatched, compatibility at the DR locus will be ranked as most important, followed by compatibility at the B locus and A locus.

Once a suitable autologous or allogeneic CB unit has been deemed an acceptable match, a sample of the CB unit will be tested for potency in the Duke STCL. If results of these tests are satisfactory and the CB unit is stored outside of Duke, the CB unit will be delivered to the Duke STCL in the frozen state.

#### 7.4 Psychological Assessments

The following measurements will be conducted with the child participant at Duke during each clinic visit:

Respondent	Measure	Domain	Length of Administration
<b>Clinician Assessments with the Child (completed at Duke)</b>	Autism Diagnostic Observation Schedule, Second Edition (ADOS-2)	ASD-Related Symptoms	45-50 minutes
	Mullen Scales of Early Learning, AGS Edition or the Differential Ability Scales, Second Edition*	Cognitive/ Language	45-60 minutes
	Expressive One-Word Picture Vocabulary Test, Fourth Edition	Cognitive/ Language	10-30 minutes
<b>Physiological and Functional Assessments of the Child (completed at Duke)</b>	Electrophysiological Response to Social and Nonsocial Stimuli	EEG	20 minutes
	Eye Gaze Tracking of Social Stimuli	ASD-Related Symptoms	10-15 minutes
<b>Clinician Observation of Parent/Child</b>	Parent-Child Interaction with Noldus EthoVision	Parent-Child Interaction	12 minutes

\* The Mullen Scales of Early Learning or Differential Ability Scales is only conducted during the baseline visit.

##### 7.4.1 Assessments for Diagnosis of Autism Spectrum Disorder:

Diagnosis of ASD will be confirmed by the DSM-5 Checklist, which will be informed by the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2) and the Autism Diagnostic Interview-Revised (ADI-R)-Shortened Version. Diagnostic evaluations will be completed by clinical research staff that have been certified as research reliable in the administration of the ADOS-2 and ADI-R.

Autism Diagnostic Interview-Revised (ADI-R), Shortened Version:<sup>37</sup> This is completed during the screening process of DukeACT, prior to the participant's arrival at Duke University and will be used to help inform a DSM-5 diagnosis of ASD. The ADI-R is a comprehensive parent interview that assesses early functioning in three domains: language/communication, reciprocal social interactions, and restricted, repetitive, and stereotyped behaviors and interests. The research reliable interviewer follows standardized procedures for obtaining information and recording responses. Interview questions include various content areas, including the participant's background, behaviors, early development and milestones, language acquisition, current communicative functioning, social development and play, interests and behaviors, and any other clinically relevant behaviors. The ADI-R shortened version follows the same protocols as the standard ADI-R, but some of the questions not required for making a diagnosis are omitted. The ADI-R shortened version takes between 90-150 minutes depending on the age of the child and the complexity of behaviors.

Autism Diagnostic Observation Schedule, Second Edition (ADOS-2):<sup>38</sup> This assessment will be used to help inform a DSM-5 diagnosis of ASD. The ADOS-2 is a standardized observational assessment of core ASD symptomatology. Age- and language-dependent modules are composed of a series of activities led by a trained, research-reliable clinician to observe the child's communication, social interactions, play, restricted, and repetitive behaviors. ADOS-2 protocols are designed to elicit behaviors that directly map onto the ASD DSM-5 criteria. The ADOS-2 can be administered to toddlers as young as 12 months of age. Age and verbal ability are used to determine the appropriate module (toddler, 1, 2, 3 or 4). For each ADOS-2 Toddler Module assessment, a score will be generated using the Toddler Module Calibrated Severity Scores developed by Esler et al. (2015) to facilitate comparison to other modules. Severity Scores will also be generated for Modules 1-4. The ADOS-2 takes around 45-60 minutes to administer. This assessment will be completed at baseline and 6 month visits.

Diagnostic Statistical Manual-5 Checklist (DSM-5): This is a clinician checklist based off of diagnostic criteria for ASD within the Diagnostic and Statistical Manual edition 5. Diagnostic criteria are taken from the DSM-5, and are utilized in order to confirm appropriate ASD diagnosis for inclusion in the study. This checklist is completed by trained clinicians using clinician's best judgment and is informed by information gathered during the administration of the ADI-R, Shortened Version and the ADOS-2. The DSM-5 takes less than 5 minutes to complete and will be completed at baseline and 6 month visits.

#### *7.4.2 Other Clinician Assessments:*

Clinical Global Impression (CGI): The CGI is a commonly used rating scale with two components that measures symptom severity and treatment response or change in clinical presentation between time points. It takes 5 minutes to complete.

The Clinical Global Impression – Severity Scale (CGI-S): The CGI-S is a 7 point scale completed at the baseline and 6-month visits that requires the clinician to rate the severity of the participant's symptoms of ASD at the time of assessment, relative to the clinician's past experience with participants who have the same diagnosis. Based on the clinician's lifetime clinical experience, a participant is assessed on severity of ASD symptoms at the time of rating - 1, normal, no symptoms; 2, borderline level of symptoms; 3, mild symptoms; 4, moderate symptoms; 5, marked symptoms; 6, severe symptoms; or 7, extremely severe symptoms. There will be three separate CGI-S ratings; these include social communicative functioning, restricted/repetitive interests and behaviors, and overall. The CGI-S will be completed at baseline and 6 month visits.

The Clinical Global Impression – Improvement (CGI-I): The CGI-I is a 7 point scale that requires the clinician to assess how much the participant's ASD symptoms have improved or worsened relative to a baseline assessment. The symptoms are rated as: 1, very much improved; 2, much improved; 3, minimally improved; 4, no change; 5, minimally worse; 6, much worse; or 7, very much worse. There will be three separate CGI-I ratings: social communicative functioning, restricted/repetitive interests and behaviors, and overall improvement. The CGI-I will be completed at the 6-month visit.

Continuous Visual Scale of Change (Visual Change): The Visual Change is completed at the 6-month visit as a measure of improvement for the key domains of impairment associated with ASD. There will be three separate scales of change completed: social communicative functioning, restricted/repetitive interests and behaviors, and overall improvement. The rater places a vertical line on the scale indicating how much he/she feels the participant has improved or worsened compared to the baseline visit, with the center of the scale indicating "No Meaningful Changes." When data is entered, the distance to the line and total length of the bar will be measured. In contrast to the CGI-S and I, it is not specifically anchored to pre-specified levels of abnormal behaviors, functional abilities or level of supports, or pre-specified "amounts of change." It is a continuous measure based on the individual clinician's judgment. The rater should base his or her judgment on the amount of change observed during various assessments and on the CGI and Visual Scales Parent Interview. Completion of the Visual Scale takes less than 5 minutes and is completed at the 6-month visit.

CGI and Visual Scales Parent Interview: The CGI and Visual Scales Parent Interview is completed by a clinician and conducted with the primary caregiver. The interview focuses on the participant's social communication abilities and challenges, restricted interests, repetitive behaviors, and overall functioning. The clinician will obtain details about frequency and quality of behaviors in different contexts, such as at home, in school, and in the community, as well as details about the level of support the child requires to function in each setting. The interview will be completed at the baseline visit and updated at the 6-month visit. The interview will take around 30 minutes to complete at the baseline visit and will take around 15 minutes to complete at the 6-month visit.

Differential Ability Scales, Second Edition (DAS-II):<sup>39</sup> The DAS-II is an assessment administered by a trained clinician to observe behavior and compute a score to assess cognitive abilities. This test is appropriate for ages 2 years, 6 months through 17 years, 11 months and will be used for participants aged 4 years, 0 months and older at baseline. This assessment takes about 45 minutes and will be conducted at the baseline visit only.

Mullen Scales of Early Learning, AGS Edition:<sup>40</sup> The Mullen Scales of Early Learning is a cognitive functioning assessment specifically designed for very young children and preschoolers, from birth to 68 months. It measures five scales of cognitive functioning: Gross Motor, Visual Reception, Fine Motor, Expressive Language, and Receptive Language (note: for DukeACT, the Gross Motor subscale will not be administered). Each scale is tested individually, and the exam is not timed. Some of the questions may require parental input in order to assess the child's ability. The exam is interactive and includes toys and manipulatives for the child to engage with during the exam. Test administration time varies from approximately 15 minutes for a one-year-old to an hour for 5-year-olds. The Mullen will be used with participants under 4 years, 0 months at the baseline visit. This assessment takes about 60 minutes and will be conducted at the baseline visit only.

Parent-Child Interaction with Noldus EthoVision: During the Parent-Child Interaction Task, Noldus EthoVision will be used. The purpose of the video tracking is to determine whether automated tracking of children's movements related to social approach or avoidance can be reliably administered and provide a valid measure of social communication in children with ASD. This paradigm is designed to automatically track movements related to social approach and avoidance behavior of children with ASD with a familiar adult. The primary dependent variable is the time spent in the periphery of a room versus near the adult. Children will be observed during two contiguous sessions conducted in the same room comprised of (1) a six-minute free-play session with toys available during which the caregiver will be silently reading a magazine in the corner of the room and (2) a six-minute parent-child interaction, where the parent joins the child for interactive play to see how the child plays with the adult and vice versa. Behavior will be recorded from a ceiling-mounted camera and software will be used to automatically track the child's movements. Dependent variables include percentage of session spent in the caregiver region of interest (ROI), latency to approach the caregiver ROI, and percentage of time spent in the periphery ROI. The Parent-Child Interaction Task will be conducted at baseline and 6 month visits.

Expressive One-Word Picture Vocabulary Test, Fourth Edition:<sup>41</sup> This is a child observation by a trained clinician that tests an individual's ability to match a spoken word with an image of an object, action, or concept. It tests an individual's ability to name, with one word, objects, actions, and concepts when presented with color illustrations. This test is appropriate for ages 2-80 years and takes about 10-30 minutes to complete; completion time is determined by an individual's verbal ability. This assessment will be conducted at baseline and 6 month visits.

Vineland Adaptive Behavior Scales (VABS) - 3 Survey Interview Form:<sup>42</sup> This will be used to assess adaptive behavior. The assessment is administered to the parent/caregiver using a semi-structured interview format. This assessment can be used with ages 0-90 years of age and takes about 60 minutes to administer. The VABS-3 is a well-standardized measure of adaptive functioning, assessing adaptive behavior in communication, daily living, socialization, motor, and maladaptive behaviors. Norms are available from birth to 90 years. The Socialization subdomain assesses play and interpersonal relationships. This assessment will be conducted at baseline and 6 month visits and will also be conducted remotely at 12 months post-baseline.

#### *7.4.3 Parent Caregiver Questionnaires:*

These questionnaires will be completed during baseline and 6 month visits at Duke and will be sent to the parent/guardian to complete remotely throughout the study. All parent questionnaires will be completed online through the survey tool Qualtrics. The Qualtrics survey tool is available for Duke users through a university-wide site license. Qualtrics is integrated with Duke's NetID authentication system but allows sharing of surveys with non-Duke users. Parents/guardians will access the Qualtrics links through a secure, personalized parent portal built by RTI International. The parent portal will be password protected.

Pervasive Developmental Disorder Behavior Inventory (PDD-BI):<sup>43</sup> The PDD-BI was developed to assess responsiveness to intervention in children with ASD. The PDD-BI is an informant-based rating scale that is designed for children 1 year, 6 mos. to 12 years, 5 mos. It assesses problem behaviors as well as appropriate social, language, and learning/memory skills. The PDD-BI assesses both social impairments typically associated with the active but odd subtype of ASD and development of pro-social skills that are integral to improved reciprocal social behavior. The PDD-BI renders raw scores as well as t-scores based on comparisons to a standardized ASD population. The PDD-BI has been validated in a PDD-BI development sample of 311 children between the ages of 1 and 17 years old. This is a parent questionnaire with 188 items that takes approximately 30-45 minutes to complete. The PDD-BI will be completed at baseline and 6-month visits and will also be completed remotely at 3, 9, and 12 months post-baseline.

Aberrant Behavior Checklist-Community (ABC-C):<sup>44</sup> This parent-completed rating scale will be used to measure aberrant behaviors associated with ASD, with an emphasis on social withdrawal. The ABC-C is a validated scale that can assess drug and other treatment effects in studies with developmentally-disabled individuals. Separate factor analyses of data from samples of institutionalized participants ( $n = 418$  [mean age 29.5 yrs.] in Stage 1 and  $n = 509$  [mean age 25.9 yrs.] in Stage 2) resulted in a 5-factor scale comprising 58 items. The factors were labeled as (1) Irritability, Agitation, Crying; (2) Social Withdrawal; (3) Stereotypic Behavior; (4) Hyperactivity, Noncompliance; and (5) Inappropriate Speech. The ABC-SQW has been used in other clinical trials focusing on the core social and communication symptoms of autism. The ABC-C has 58 items, and each item is rated as 0= not at all a problem, 1= the behavior is a problem, but slight in degree, 2= the problem is moderately serious, or 3= the problem is severe in degree. This parent-completed rating scale takes approximately 10 to 15 minutes to complete. The ABC-C will be completed at baseline and 6 month visits and will be completed remotely at 3, 9, and 12 months post-baseline.

Intervention History Questionnaire: This questionnaire is completed by a primary caregiver to obtain detailed information on behavioral health interventions that the child/family has been involved in over the past 3 months or since the questionnaire was last administered. Information is collected about the type and quantity of interventions, services, and treatments the child is receiving. This questionnaire will be administered on a monthly basis.

Behavior Rating Inventory of Executive Function-Preschool Version (BRIEF-P):<sup>45</sup> This assessment is a questionnaire for parents of preschool-aged children that enables professionals to assess executive function behaviors in the home and preschool environments. It is designed for a broad range of preschool children including those with emergent learning disabilities and attentional disorders, language disorders, traumatic brain injuries, lead exposure, pervasive developmental disorders, and other developmental neurological, psychiatric, and medical conditions. The BRIEF-P contains 63 items and takes about 15 minutes to complete. The BRIEF-P will be used with participants under the age of 5 years at baseline. This questionnaire will be completed at baseline and 6 month visits and remotely 12 months post-baseline.



Behavior Rating Inventory of Executive Function (BRIEF):<sup>46</sup> This assessment is a questionnaire for parents of school age children that enables professionals to assess executive function behaviors in the home and school environments. The parent form of the BRIEF contains 86 items within eight theoretically and empirically derived clinical scales that measure different aspects of executive functioning: Inhibit, Shift, Emotional Control, Initiate, Working Memory, Plan/Organize, Organization of Materials, and Monitor. The BRIEF takes about 15 minutes to complete and will be used with participants aged 5 years and older at baseline. This questionnaire will be completed at baseline and 6 month visits and remotely 12 months post-baseline.

Sensory Experiences Questionnaire, Version 2.1 (SEQ):<sup>47,48</sup> The SEQ 2.1 asks parents to respond, on a 5-point Likert scale, to 45 questions about the frequency of their children's responses to sensory stimuli in the context of daily activities and routines. This study will use a modified version that only asks parents to complete the Likert scale questions and omits all open-ended questions. The SEQ 2.1 has been validated for children with autism ages 2 -12 years, been shown to discriminate children with ASD from developmental delay and typically developing controls, and has a high internal consistency ( $\alpha = 0.80$ ).<sup>49</sup> Summary scores will be derived for hyper-responsiveness (SOR). The SEQ 2.1 takes around 10 minutes to complete and will only be completed at the baseline visit.

Early Life Exposures Assessment Tool (ELEAT): This is a parent questionnaire that assesses for a multitude of environmental exposures that the parents/child may have been exposed to in the child's early life and development. The questionnaire asks a variety of questions about life before pregnancy, during pregnancy, and during the child's first year of life, and gives a very detailed, quantitative look at any possible food or chemical exposures that may have been harmful. A modified and shortened version of the ELEAT which only includes relevant variables will be used for the study. This assessment takes about 10 minutes to complete and will only be completed at the baseline visit.

Pediatric Quality of Life Inventory (PedsQL) Gastrointestinal Symptoms Scales:<sup>50</sup> The PedsQL Gastrointestinal Symptoms Scales is a 10 minute parent questionnaire that measures gastrointestinal symptoms in children as over 50% of children with ASD suffer from gastrointestinal discomfort. This assessment will be completed at baseline and 6 month visits and remotely 12 months post-baseline.

Demographic information will be obtained at the baseline visit for each participant using the Duke Center for Autism and Brain Development Demographics Form. Interval medical and social histories will be obtained at the six-month visit in person and with the aid of interval history questionnaires.

#### *7.4.4 Other Assessments:*

The Visit Preparation Inventory is a brief questionnaire that is completed with the parent prior to the child's visit. Information about the child's preferences and dislikes is assessed on this form that is then utilized to prepare reinforcement techniques for the child's visit. In addition, sensory sensitivities related to the completion of EEG or eye tracking are

assessed in order to best prepare for the child's EEG sessions. This questionnaire is completed by the clinical research coordinator during a phone call with the caregivers one to two weeks before baseline and 6 month visits.

## **7.5 Neurophysiology and Neuroimaging**

### *7.5.1 Neurophysiology:*

Electroencephalography (EEG): EEG is a non-invasive measure of brain activity. A flexible dense array of electrodes (EGI System 400, Electrical Geodesics Inc., Eugene, Oregon) is placed over the head and secured with a chin strap. EEG signals are amplified and sent to recording computer for on-line viewing. EEG is used here to investigate patterns of brain activity elicited by social and non-social stimuli. The EEG takes around 20 minutes to complete and will be completed at baseline and 6 month visits.

EEG will be used to assess changes in cortical activation (as reflected by changes in alpha, theta, and beta rhythms) and functional connectivity (as reflected by changes in EEG coherence) during a baseline condition and while viewing social and non-social stimuli. Alpha oscillations have been shown to emerge from activations of the thalamo-cortical network and have also been demonstrated to be present in subcortical areas, including the hippocampal region. Alpha frequencies result from a reciprocal interplay between excitatory and inhibitory neurons and are influenced by cholinergic, serotonergic, and glutamatergic mechanisms. Alpha activity increases during a relaxed state and decreases during active stimulus processing. Theta oscillations are especially prominent in the hippocampal region, are influenced by the interaction between glutamatergic and gamma-aminobutyric acidergic (GABAergic) neurons, and may correlate with synaptic plasticity.

EEG will be recorded during a resting baseline state and while viewing standardized videotaped segments of social (female singing a nursery rhyme) and nonsocial (activated toys) stimuli. In a study of preschool aged children with ASD, alpha and theta EEG power during viewing of social and nonsocial stimuli have been shown to change as a function of behavioral treatment and are specifically correlated with improvements in social behavior.<sup>51</sup> EEG data will be recorded from 128-channel dense electrode array Geodesic sensor nets (recorded online with reference to the vertex) at 500 Hz, high-pass filtered at 0.1 Hz and low-pass filtered at 200 Hz. EEG will be edited both through automatic artifact-detection software (Net Station 4.4) and hand-editing without knowledge of group membership.

Spectral analyses. EEG data will be Fourier-transformed using Welch's method (implemented in Matlab, R2012b). Power estimates will be averaged across electrode groups (right posterior and anterior, left posterior and anterior, midline occipital electrodes) and natural log-transformed to reduce skew. Planned analyses contrasted power in the theta (5-7 Hz) and alpha (9-11 Hz) bands during the presentation of face versus object stimuli, allowing the analyses to account for individual differences in absolute power. Alpha and theta power will be analyzed using repeated measures ANOVAs, with stimulus (face, object) and region as within-participant variables.

Eye Tracking of Social Stimuli (EGT): The EGT is a 15 minute technical assessment using specialized equipment in a controlled setting to track visual attention via eye movements of children with autism. Children with ASD glean useful social information from different parts of the observed face as compared to a control group of children. This assessment is appropriate for children of 1 year to adult. The children will be shown videotapes of scenes involving social and nonsocial stimuli. The depending variables include how much time the child spends looking at the social versus non-social stimuli in the videotapes, including the specific aspects of the social stimuli that attract and hold the child's attention. The EGT will be conducted at baseline and 6 month visits.

#### *7.5.2 Neuroimaging:*

MRI will be used to assess potential structural/anatomic modifications, and functional changes, in the brain before and after cell therapy. MRI images will also be used to correlate brain iron with ASD symptoms. Changes in MRI images may reflect alterations in astrocyte activity, blood perfusion, fiber tract integrity, brain network integrity, myelin microstructural integrity and brain functional connectivity. The following imaging will be utilized:

- High-Resolution T1 Imaging, 3D IR-prepped FSPGR, 1x1x1 mm resolution, estimated imaging time 3 min.
- High-resolution perfusion imaging, 3D spiral with arterial spin labeling, target resolution 1.5x1.5x1.5 mm (will evaluate 1x1x1 provided SNR is sufficient), estimated imaging time 3 min.
- High-resolution diffusion tensor imaging, 2D multi-shot DW EPI base sequence, 25 diffusion encoding directions, b factor 800 s/mm<sup>2</sup>, target resolution 1.5x1.5x1.5 mm (will evaluate 1x1x1 provided SNR is sufficient), estimated imaging time 10 min.
- High-resolution fcMRI, 2D single- or two-shot EPI base sequence, target resolution 1.5x1.5x1.5 mm (will evaluate 1x1x1 provided SNR is sufficient), estimated imaging time 10 min. (5 min. per run, two runs).
- High-resolution quantitative susceptibility imaging, multi-echo 3D FSPGR based acquisition, target resolution 1x1x1 mm, estimated imaging time 5 min.

### **7.6 Biological Samples**

The following samples will be obtained for future biomarker studies (see Appendix 2 for additional details):

- Blood for immunological, DNA, and RNA studies
- Blood for generation of induced pluripotent stem cells (iPSCs)
- Urine
- Saliva
- Parental blood samples

### **7.7 Study Product Infusion**

On the day of infusion, CB cells or placebo product will be prepared by the STCL and provided for infusion of the patient in the outpatient clinic under the supervision of the

study team and Pediatric Blood and Marrow Transplant Program staff. A peripheral IV will be placed by clinical staff, anesthesia or a member of the study team. Prior to the study infusion, premedications (Benadryl and Solumedrol) will be administered. The infusion will be given over approximately 2-30 minutes using standard practices. The child will receive 1-1.5x maintenance IV fluids as described below and be observed in the clinic for a minimum of 1 hour after the infusion. Patients will be discharged from clinic after at least 1 hour providing all vital signs are at their baseline and they are awake and asymptomatic with no evidence of toxicity. Patients will be evaluated by study staff the day after the infusion to assess for any infusion-related adverse reactions or complications. A phone call to parents/guardians by study staff to assess safety of the infusion will be conducted 10 days to 2 weeks after the infusion.

Maintenance IV Fluid Rate (Holliday-Segar Method from Harriet Lane Handbook)

<u>Body weight:</u>	<u>mL/kg per day</u>	
1st 10 kg	100	divided by 24hr/day
2nd 10 kg	50	divided by 24 hr/day
each add'l kg	20	divided by 24 hr/day

If a patient has evidence of illness on the day of planned infusion, including but not limited to fever  $>38.5^{\circ}\text{C}$ , vomiting, diarrhea, or respiratory distress, the infusion will be postponed.

## **7.8 Care During Unexpected Events**

In the event that a patient develops signs or symptoms of anaphylaxis including urticaria, difficulty breathing, cough, wheezing, or vomiting during their CB infusion, the infusion will be terminated and appropriate medical therapy initiated.

## 7.9 Required Evaluations

### 7.9.1 Medical and Safety Assessments:

	Time Points <sup>#</sup>							
	Screening	Baseline (Visit 1)	2 weeks post-infusion	3 mo.	6 mo. (visit 2)	2 weeks post-infusion	9 mo.	12 mo. <sup>&amp;</sup>
<b>CBCD*, CMP*, Type &amp; Screen*, patient HLA</b>	X							
<b>CBU potency; HLA confirmatory typing</b>	X							
<b>Review of prior records ± videos</b>	X							
<b>History &amp; Physical</b>		X			X			
<b>Donor Referral Panel</b>		X			X			
<b>Patient DNA sample for chimerism</b>		X						
<b>Donor DNA sample for chimerism<sup>+</sup></b>		X <sup>+</sup>			X <sup>+</sup>			
<b>CBCD*, CMP*, Type &amp; Screen*, Direct Coombs, HLA Antibody Screen (PRA), ESR, CRP, Ferritin, Immune Reconstitution Panel, Humoral Immune Profile</b>		X			X			X
<b>In-person neuropsychological evaluation</b>		X			X			
<b>Remote psychological evaluation</b>				X			X	X
<b>Brain MRI</b>		X			X			
<b>Peripheral blood chimerism (donor CB recipients only)</b>					X			X
<b>CB infusion (TNCC, viability, CD34<sup>+</sup> cells, CFU, sterility cultures)</b>		X			X			
<b>Safety Assessment – in-person evaluation</b>		X (Day after infusion)			X (Day after infusion)			
<b>Safety Assessment – phone call/survey</b>			X	X	X	X	X	X

\* CBCD, CMP, and Type & Screen may be obtained at initial visit or within 6 months prior to enrollment

&12 month laboratory evaluations may be obtained remotely or mailed to Duke

<sup>+</sup>Donor DNA will be obtained at the time of CB thaw

<sup>#</sup>Safety and return assessments should be performed within a month of the indicated time point.

### 7.9.2 Diagnostic, Behavioral, Neurocognitive, and Neurophysiological Evaluations:

	Measure	Length	Time Points <sup>#</sup>				
			Baseline (visit)	3 mo. (remote)	6 mo. (visit)	9 mo. (remote)	12 mo. (remote)
<b>Pre-Visit Clinician Assessment w/Parent</b>	Autism Diagnostic Interview, Revised, Shortened Version (ADI-R)	90-150 minutes	X (pre-visit)				
<b>Clinician Assessment w/Child</b>	Autism Diagnostic Observation, Second Edition (ADOS-2)	45-60 minutes	X		X		
	Mullen Scales of Early Learning, AGS Edition	60 minutes	X				
	Differential Ability Scales, Second Edition (DAS-II)	45 minutes	X				
	Parent-Child Interaction (proximity seeking)	12 minutes	X		X		
	Expressive One-Word Picture Vocabulary Test, Fourth Edition (EOWPVT)	10-30 minutes	X		X		
<b>Physiological and Functional Assessments w/Child</b>	Electrophysiological Response to Social Stimuli (EEG)	20 minutes	X		X		
	Eye Gaze Tracking of Social Stimuli (EGT)	10-15 minutes	X		X		
<b>Clinician Assessments w/Parent</b>	Vineland Adaptive Behavior Scales, Third Edition, Survey Interview Form	60 minutes	X		X		X
<b>Parent Questionnaires</b>	Pervasive Developmental Disorder-Behavior Inventory (PDD-BI)	30 minutes	X	X	X	X	X
	Intervention History*	15 minutes	X	X	X	X	X
	The Early Life Exposures Assessment Tool (ELEAT)	10 minutes	X				
	Aberrant Behavior Checklist-Community	10 minutes	X	X	X	X	X
	Sensory Experiences Questionnaire 2.1	10 minutes	X				
	Brief Rating Inventory of Executive Functioning or Brief Rating Inventory of Executive Function-Preschool	15 minutes	X		X		X
	PedsQL-Gastrointestinal Symptoms Inventory	10 minutes	X		X		X
<b>Other Clinician Assessments</b>	Diagnostic Statistical Manual 5-Checklist (DSM-5)	3-5 minutes	X		X		
	Clinical Global Impression-Severity and Improvement and Visual Scales	15 minutes	X		X		

\*Intervention history will be collected on a monthly basis.

<sup>#</sup>Assessments should be performed within a month of the indicated time point.

## 8.0 STATISTICAL CONSIDERATIONS

This study will enroll autistic children age 2 to 7 years of age. Participants will be randomized in a 2:1 allocation to cord blood or placebo with stratification by important prognostic factors (see below for randomization plan). The primary objective of the study will be fulfilled by comparing the mean of the 6-month change in VABS-3 socialization domain standard score between cord blood and placebo arms. The study also includes pre-planned subgroup analyses comparing the same outcome between treated patients receiving autologous or allogeneic cord blood vs. placebo, and between patients receiving

autologous and allogeneic cord blood. The approach to sample size estimation was based on the primary comparison of cord blood to placebo. Power for the pre-planned subgroup analyses is described using the sample size planned for the primary comparison.

Note: All children in the placebo arm will receive cord blood after reaching their six-month evaluation. This serves simultaneously as a recruitment tool and to inform exploratory analyses. However, sample size and power calculations are based only on the planned comparisons between patients assigned cord blood vs. placebo at six months.

## **8.1 Study Design**

This is a single site, prospective, randomized, double-blind, parallel group study of a single intravenous autologous or allogeneic, unrelated CB infusion in children ages 2-7 years with ASD. Participants will be randomly assigned to *Sequence A*, consisting of a single infusion of CB cells at baseline followed 6 months later by a single infusion of placebo, or *Sequence B*, consisting of an infusion of placebo at baseline followed 6 months later by an infusion of CB cells. All participants will be treated with CB cells; participants with an available qualified autologous CB unit will receive autologous cells, and those without suitable autologous CB available will receive  $\geq 4/6$  HLA-matched, allogeneic, unrelated donor CB cells from the Carolinas Cord Blood Bank. All infusions will be double-blinded. The primary outcomes will be assessed 6 months after the initial infusion in the sequence. Duration of study participation will be 12 months from the time of baseline infusion.

## **8.2 Accrual**

It is estimated that up to 4 research participants will be enrolled each week and that approximately 15 months of accrual will be necessary to enroll 190 participants.

## **8.3 Study Duration**

Research participants will be followed for safety for 6 months after the second study infusion.

## **8.4 Demographics and Baseline Characteristics**

Demographics and baseline characteristics will be summarized for all research participants. Characteristics to be examined include age, sex, race/ethnicity, and baseline behavioral status.

## **8.5 Description of the Primary Outcome Measure**

The primary objective of the study is to determine, in a randomized, placebo controlled, best available donor source trial, the efficacy of a single intravenous infusion of umbilical CB in improving the core symptoms of children with ASD. Because there is no single best outcome measure recommended for use in interventional clinical trials of autism,<sup>52</sup> a pilot study (the DukeABC trial) was conducted that enrolled 25 children with ASD and evaluated the utility of several measures for documenting change in ASD symptoms over a short time period post-treatment with cord blood. In the DukeABC trial, clinically and statistically significant change was observed over a 6-month period on several outcome measures that quantify ASD core symptoms, including the VABS-II, PDDBI, and CGI. The VABS-3 socialization domain standard score was selected as the primary outcome measure in this trial as it assesses a core autism symptom, social communication that is

hypothesized to be affected by the therapy under study. The PDD-BI and CGI are included in this study as secondary outcomes.

#### **8.6 Description of the VABS-3 Socialization Domain Standard Score**

The VABS-3 will be administered to the participant's parents by interview in this study. The VABS-3 measures adaptive behavior in several domains including socialization, which is the domain used as the primary outcome measure in this trial. The socialization domain is a summary of measures in three subdomains: interpersonal relationships, play and leisure time, and coping skills. Raw scores within these subdomains are standardized to V-scale scores for each domain (mean=15, SD=3). The sum of the subdomain V-scale scores are then standardized to a normal distribution with mean 100 and standard deviation 15, thus resulting in the socialization standard score. Our study will use the difference between this standard score measured at baseline and 6-months post-treatment. Positive change over a six-month period indicates gains in socialization behaviors. The median change in socialization standard score over a six-month period (n=24) in the DukeABC trial was 2 points (range: -8 to 30) and this was significantly different from zero (P=0.02, Wilcoxon signed rank test). Children with non-verbal IQ less than 55 (n=9) had a median change of -1 point (range: -8 to 14) whereas children with non-verbal IQ  $\geq 55$  (n=15) had a median change of 6 points (range: -6 to 30) (P=0.02, Wilcoxon rank sum test). No association was observed between the change score and sex (P=.07, Wilcoxon rank sum test, n=4 females) or baseline age (Spearman  $r = -0.18$ , P=0.41).

#### **8.7 Sample Size and Power Calculations**

We estimated the sample size required for 80% power to detect various standardized effect sizes comparing patients randomized to cord blood vs. placebo. The Type I error rate was fixed at 2.5% for this comparison to account for the additional pre-planned comparisons described in later sections.

The null and alternative hypotheses for the primary comparison are:

$$\begin{aligned}H_0: \mu_{CB} - \mu_P &= 0 \\H_A: \mu_{CB} - \mu_P &\neq 0\end{aligned}$$

Where  $\mu_{CB}$  is the population mean 6-month change in VABS II socialization standard score in autistic children treated with cord blood and  $\mu_P$  is the analogous mean in autistic children treated with placebo.

Sample size was estimated assuming a two-sample, equal variance t-test. Calculations were done using PASS 12.0 using non-central t-distributions. The table below shows the sample sizes required for 80% to detect various standardized effect sizes assuming  $\alpha=0.025$  and 2:1 allocation to cord blood or placebo.



Actual Power	N <sub>Placebo</sub>	N <sub>Cord Blood</sub>	N <sub>Total</sub>	Cohen's <i>d</i>
<b>0.80055</b>	357	714	1,071	0.20
<b>0.80145</b>	229	458	687	0.25
<b>0.80201</b>	160	320	480	0.30
<b>0.80284</b>	118	236	354	0.35
<b>0.80023</b>	90	180	270	0.40
<b>0.80454</b>	72	144	216	0.45
<b>0.80094</b>	58	116	174	0.50
<b>0.80018</b>	48	96	144	0.55
<b>0.80588</b>	41	82	123	0.60
<b>0.80510</b>	35	70	105	0.65
<b>0.80073</b>	30	60	90	0.70
<b>0.81320</b>	27	54	81	0.75
<b>0.81617</b>	24	48	72	0.80
<b>0.80871</b>	21	42	63	0.85
<b>0.81275</b>	19	38	57	0.90
<b>0.80900</b>	17	34	51	0.95
<b>0.82502</b>	16	32	48	1.00

A total sample size of 144 (96 on cord blood and 48 on placebo) will provide 80% power to detect a moderate ( $d=0.55$ ) sized treatment effect given the stated design parameters. Expansion of the sample size by ~15% to allow for dropout, and to facilitate piloting the study procedures with the first 2-3 enrolled subjects yields an approximate sample size of 165 participants (110 on cord blood and 55 on placebo). **Therefore, with a sample size of 165 participants allocated 2:1 to cord blood: placebo, the trial will have greater than 80% power to detect a moderate sized treatment effect with strict control of Type I error.** However, in recognition of the potential need to modify the sample size to maintain power for the planned alternative hypothesis (see Section 8.13, Interim Analysis) we set an anticipated maximum of 190 participants.

## 8.8 Power for Subgroup Analyses

In this study it is of interest to compare treatment effects by best available cell source. However, the actual power available for the requisite subgroup analyses is dependent upon the size of the groups, which cannot be known *a priori*. Therefore, we adopted a descriptive approach to power analysis for these comparisons under what we believe to be likely accrual eventualities, and using a simplified analytical model.

To explore power and sample size we selected a 2-way ANOVA framework where the main effects were treatment (cord blood or placebo) and cell source (autologous or allogeneic). Within this model the investigation of cell source is formulated as the F-test of interaction in the 2-way ANOVA, which compares the treatment effect by cell source. This test uses the Bonferroni-corrected alpha specified in the primary analysis (0.025). A primary assumption of this model is that group sizes are equal. Given the design of our trial this is unlikely to occur. Nonetheless, illustration of detectable effect sizes with balanced designs can be instructive as they provide the “best case scenario.” Given a fixed

power, sample size, and Type I error the actual detectable effect size will be larger in unbalanced designs than balanced designs. Therefore, we estimated the detectable effect size under different sized balanced designs to describe a possible range of detectable effect sizes in our trial.

We began by calculating power assuming a balanced design with an average of 41.25 patients per group (the planned accrual of 165 patients divided among four groups defined by cord blood/placebo and autologous/allogeneic) and a common main effect and interaction effect size. Using PASS version 12.0 we estimated that with this sample size and the aforementioned design parameters our study would have approximately 80% power to detect a standardized effect size of 0.25 (considered a medium ANOVA effect size by Cohen) for the interaction test. Using an average sample size that is equal to the smallest expected group sample size in our trial ( $n=27.5$ , assuming the placebo group is evenly divided by cell source) the detectable effect size is 0.33, which is closer to the large (0.4) effect size defined by Cohen.

Therefore, we can be confident that our trial will be able to detect moderate to large differences in the treatment effect by cell source with strict control of Type I error using an unbalanced 2-way ANOVA.

#### **8.9 Correction for Multiple Testing**

Our sample size plan incorporates the Bonferroni adjustment for control of Type I error across the pre-planned statistical tests involving the primary endpoint; i.e., the test of cord blood vs. placebo and the test of interaction that evaluates treatment effect by cell source.

Secondary endpoints will be tested assuming a per-comparison alpha of 0.05. The False Discovery Rate (FDR) procedure will then be applied to determine which results are significant after consideration for multiple testing. The primary endpoint comparison of cord blood vs. placebo will be included in the FDR procedure.

Further details are available in the Statistical Analysis Plan (SAP).

#### **8.10 Randomization Plan**

The study will randomize participants to cord blood or placebo with stratification by age ( $< 5$  vs.  $\geq 5$  years), non-verbal IQ ( $< 55$  vs.  $\geq 55$ ), and best available cell source (autologous vs. allogeneic) and will utilize a randomly varying block size with 2:1 allocation to cord blood and placebo. The DukeABC trial showed that IQ was a likely confounder of the change in Vineland socialization standard score over a 6-month period. Age is also a likely confounder as children's developmental rate varies with age. The availability of autologous or allogeneic cord blood prior to randomization is a potential confounder as it might correlate with socioeconomic status, and this may influence the use of therapy outside the trial. Although there is a dearth of efficacious therapies for improvement of the core symptoms of autism, the use of therapy outside the trial may result in a tendency of parents to over-report positive outcomes on the Vineland-3 or the PDD-BI. Thus, by stratifying our randomization on available cell source we will preserve the validity of our comparison of the treated and cord blood groups.

### **8.11 Analysis Plan**

Full details of the planned analysis are available in the accompanying Statistical Analysis Plan (SAP). Briefly, safety analyses will be primarily descriptive and will rely on graphical displays and tabulated descriptive statistics. The primary efficacy analysis comparing cord blood to placebo is based on a two-sample t-test of the mean difference (from baseline to 6 months) in the VABS 3 socialization domain standard score comparing the cord blood and placebo arms. Study procedures will be piloted in the first 2-3 enrolled subjects and therefore these subjects may not be included in the primary analysis. Additional analyses are described in the SAP.

#### *Analysis Populations:*

##### Full Analysis Population

This population constitutes all enrolled participants and is used for descriptive purposes; e.g. to identify subjects who are not evaluable for the primary efficacy analysis.

##### Modified Intention to Treat (mITT) Population

This analysis set will include only subjects who complete all of the baseline and follow-up VABS-3 socialization domain standard score. Subjects who are missing one or both of these evaluations cannot contribute to the primary efficacy analysis because the change score on the VABS-3 socialization domain is not observable. Subjects will be analyzed according to the treatment they were assigned (regardless of what they actually receive).

##### Safety Population

The safety population will include all subjects who received at least 1 infusion. Analyses of the Safety Population will be conducted using an as-treated approach, which considers each patient according the treatment actually received rather than the treatment they were assigned.

Each subject's status with regard to each analysis population will be determined prior to breaking the blind.

### **8.12 Loss to Follow-up and Discontinuation**

Patients who are lost to follow-up or are discontinued from the study will not be replaced, and additional participants will not be randomized.

### **8.13 Interim Analysis**

A blinded re-estimation of sample size will be conducted when the primary endpoint is evaluable on approximately 75% of the participants.

## **9.0 SAFETY AND ADVERSE EVENT REPORTING**

### **9.1 Definitions**

Adverse Event (AE): An adverse event is any untoward medical occurrence associated with the use of the investigational product regardless of whether it is considered related to the investigational product.

*Serious Adverse Event (SAE)*: An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

*Grade/Severity*: Grade/severity will be assessed according to CTCAE v4.0 guidelines.

*Suspected Adverse Reaction*: A suspected adverse reaction is any adverse event for which there is a reasonable possibility that the investigational product caused the adverse event. “Reasonable possibility” means there is evidence to suggest a causal relationship between the investigational product and the adverse event.

*Causality*: The investigator will use the following question when assessing causality of an adverse event to the investigational product: “Is there a reasonable possibility that the investigational product caused the event?” An affirmative answer designates the event as a suspected adverse reaction.

## **9.2 Adverse Event Reporting**

All AEs reported or observed during the study beginning at the time of the study infusion must be recorded. Information to be reported includes when the site became aware of the event, investigator-specified assessment of severity and relationship to study therapy, whether there is an alternative etiology, seriousness, any treatment or evaluations, and outcome. In general, investigators should report AEs as diseases or syndromes whenever possible, instead of reporting individual component symptoms, signs, laboratory abnormalities, and sequelae.

SAEs (fatal, life-threatening or requiring hospitalization) will be reported within 7 calendar days of receipt of the information. All fatal or life threatening SAEs will be reported by the investigator/representative to the FDA by phone or fax within 7 calendar days after receipt of the information, following FDA guidelines. All serious and unexpected AEs will be reported to the FDA via a written report within 15 days of receipt of the information (21 CFR 312.32). If the principal investigator assesses an event to be unrelated to the study, then the event will not require expedited reporting but will be included in the annual summary report.

## **9.3 Stopping Guidelines**

Stopping guidelines based on safety will be monitored by the CRO and will be used to indicate boundaries requiring discussion by the investigators and DSMB. The study will be stopped for a safety review if:

- Any participant experiences a grade 4-5 infusion reaction within 48 hours of infusion;

- OR
- Two or more grade 4-5 adverse events determined to be temporally related by the medical safety monitor and/or the DSMB occur;
- OR
- Any participant experiences a blood stream infection within 6 months of infusion;
- OR
- Any participant develops grade II-IV GvHD;
- OR
- Any death.

A consensus decision to stop the study will be made by the investigators and the DSMB. Such a decision with its supporting documentation and possible future plans for the study will be submitted to, and discussed with, the FDA.

#### **9.4 Participant Replacement**

The sponsor may replace any participant who has not been dosed.

#### **9.5 Emergency Unblinding**

All participants will be treated with CB infusion, but the time course will vary between groups and participants will be blinded to the order in which they receive CB and placebo infusions. If an SAE occurs and is related to either the study product, the participant and family will be unblinded to the infusion that they received. Documentation of unblinding will be indicated on the CRF and on the SAE report if one is required.

### **10.0 DATA SAFETY MONITORING BOARD (DSMB)**

A DSMB will be formed and a charter established. Members of the DSMB will be independent of Duke University and comprised of a minimum of three members, including a clinician with experience in the treatment of ASD and a physician with experience in cell therapy. The DSMB will be notified immediately for all unexpected SAEs directly related to the study product throughout the study. Interim reports of safety and tolerability information will be prepared and will be forwarded to the DSMB for review three months after 50 participants have received their initial infusion. Policies of the DSMB will be described in the DSMB charter and signed by all members.

All study related and unexpected SAEs reported or observed during the study beginning at the time of the study infusion must be recorded and maintained in the study participant's paper files. Severe adverse infusion reactions (fatal, life threatening or requiring hospitalization) will be reported to the IRB and FDA in accordance with HRPP policies.

### **11.0 DATA HANDLING AND QUALITY ASSURANCE**

#### **11.1 Case Report Forms**

As part of the responsibilities assumed by participating in the study, the Principal Investigator or Sub-Investigators agree to maintain adequate case histories of the research participants treated as part of the research under this protocol. The Principal Investigator

or Sub-Investigator agrees to maintain accurate CRFs and source documentation as part of the case histories. Duke University will supply the CRF electronically (eCFR) through secured electronic data entry systems.

### **11.2 Video and Audio Recordings**

Video recordings of potential participants from parents and guardians may be submitted and used for determining study eligibility. Audio and video recordings may also be obtained of portions of the evaluations and interviews if indicated with parental consent, and may include full facial features. The recordings will be used solely for analysis by the research team or for educational purposes if written consent is obtained from the parent/guardian. They will be stored electronically on a password-protected server and identified by the participants' study ID.

### **11.3 Inspection of Records**

The Principal Investigator or Sub-Investigators and institutions involved in the study will permit study-related monitoring, audits, IRB review, and regulatory inspection(s) by providing direct access to all study records. In the event of an audit, the Principal Investigator or Sub-Investigator agrees to allow the Food and Drug Administration (FDA), or other regulatory agency access to all study records. The Principal Investigator or Sub-Investigators should promptly notify all relevant parties of any audits scheduled by any regulatory authorities and promptly forward copies of any audit reports received to the both.

### **11.4 Study Record Retention**

Study results will be retained in the patient's research record for six years after the study is completed or until the patient reaches the age of 21, whichever is longer. Essential documents should be retained until at least two years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or at least two years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period, however, if required by the applicable regulatory requirements.

## **12.0 ADMINISTRATIVE ASPECTS**

The following administrative items are meant to guide the Principal Investigator or Sub-Investigator in the conduct of the study but may be participant to change based on industry and government Standard Operating Procedures or Working Practice Documents or Guidelines.

### **12.1 Confidentiality**

All laboratory specimens, evaluation forms, reports, and other records will be identified in a manner designed to maintain research participant confidentiality. All records will be kept in a secure storage area with limited access. Clinical information will not be released without the written permission of the research participant's guardian except as necessary for monitoring and auditing.

The Principal Investigator or Sub-Investigator and all employees and coworkers involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study.

## **12.2 Institutional Review Board Approval**

Federal regulations and the ICH guidelines require that approval be obtained from an IRB prior to participation of human research participants in research studies. Prior to the study onset, the protocol, informed consent, any advertisement used to recruit study patients, and any other written information regarding this study to be provided to the research participant or the research participant's legal guardian must be approved by the IRB.

All IRB approvals should be signed by the IRB Chairman or designee and must identify the IRB name and address, the clinical protocol by title and/or protocol number, and the date the approval and/or favorable opinion was granted.

The Principal Investigator or Sub-Investigator is responsible for obtaining continued review of the clinical research at intervals not exceeding one year or otherwise specified by the IRB. The Principal Investigator or Sub-Investigator must supply the Sponsor or its designee with written documentation of continued review of the clinical research.

## **12.3 Modification of the Protocol**

Any changes in this research activity, except those necessary to remove an apparent, immediate hazard to the research participant, must be reviewed and approved by the IRB.

## **12.4 Informed Consent**

A written informed consent in compliance with Part 50 of Title 21 of the Code of Federal Regulations (CFR) and Institutional IRB shall be obtained from each research participant prior to entering the study or performing any unusual or non-routine procedure that involves risk to the research participant.

Before enrollment, each prospective research participant and/or his/her legal guardian will be given a full explanation of the study and be allowed to read the approved informed consent form. Once the Principal Investigator or Sub-Investigator is assured that the research participant/legal guardian understands the implications of participating in the study, the research participant/legal guardian will be asked to give consent to participate in the study by signing the informed consent form.

The Principal Investigator or Sub-Investigator shall provide a signed/dated copy of the signed informed consent to the research participant and/or legal guardian.

## **12.5 Protocol Violations and Deviations**

The Principal Investigator or Sub-Investigator or designee must document and explain in the research participant's source documentation any deviation from the approved protocol. The Principal Investigator or Sub-Investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard to study research participants without prior IRB approval. As soon as possible after such an occurrence,

the implemented deviation or change, the reasons for it, and any proposed protocol amendment(s) should be submitted to the IRB for review and approval, to the Sponsor for agreement, and to the regulatory authorities, if required.

A deviation from the protocol is an unintended and/or unanticipated departure from the procedures and/or processes approved by the Sponsor and the IRB and agreed to by the Principal Investigator or Sub-Investigator. Deviations usually have an impact on individual research participants or a small group of research participants and do not involve inclusion/exclusion or primary endpoint criteria. A protocol violation occurs when there is nonadherence to the protocol that results in a significant, additional risk to the research participant, when the research participant or Principal Investigator or Sub-Investigator has failed to adhere to significant protocol requirements (inclusion/exclusion criteria) and the research participant was enrolled without prior Sponsor approval, or when there is nonadherence to FDA regulations and/or ICH GCP guidelines.

Protocol violations and deviations will be documented by the clinical monitor throughout the course of monitoring visits. Principal Investigators or Sub-Investigators will be notified of violations and/or deviations in writing by the monitor. The IRB should be notified of all protocol violations and deviations in a timely manner as required by the site's IRB.

#### **12.6 Study Reporting Requirements**

By participating in the study, the Principal Investigator or Sub-Investigator agrees to submit reports of serious adverse events according to the timeline and method outlined in the protocol. In addition, the Principal Investigator or Sub-Investigator agrees to submit annual reports to his/her IRB as appropriate. The Principal Investigator or Sub-Investigator also agrees to provide the Sponsor with an adequate report shortly after completion of the Principal Investigator's or Sub-Investigator's participation in the study.

#### **12.7 Financial Obligations**

Duke University is not financially responsible for further testing/treatment of any medical condition that may be detected during the screening progress. In addition, in the absence of specific arrangements, Duke University is not financially responsible for further treatment of the research participant's disease.

#### **12.8 Study Conduct**

The Principal Investigator agrees that the study will be conducted according to the principles of the ICH E6 Guideline on GCP and the principles of the World Medical Association Declaration of Helsinki. The Principal Investigator will conduct all aspects of this study in accordance with all national, state, and local laws or regulations.

#### **12.9 Publications**

Following completion of the study, the data may be considered for reporting at a scientific meeting and/or for publication in a scientific journal. In these cases, Duke University will be responsible to determine how the manuscript is written and edited, the number and order of authors, the publication to which it will be submitted, and other related issues.



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## 14.0 APPENDIX 1: SAFETY QUESTIONNAIRE

### BASELINE MEDICAL & BEHAVIORAL HISTORY QUESTIONNAIRE

(To be completed on Monday of the baseline visit)

**Please tell us if your child has ever experienced any significant problems with any of the following conditions using the column marked EVER. If so, tell us whether they have continued to experience significant problems with that condition during the past 2 months in the next column.**

		EVER		Past 2 months	
		No	Yes	No	Yes
1	Has your child ever had any fevers with no clear cause?				
2	Has your child ever had any serious or recurrent infections?				
3	Has your child ever had any allergic reactions?				
4	Has your child ever had significant problems with autoimmune disorders?				
5	Has your child ever had any significant problems with rashes or abnormal skin lesions?				
6	Has your child ever had jaundice (yellowing of the skin or eyes), except as a newborn?				
7	Has your child ever received a blood transfusion?				
8	Has your child ever had any significant problems with bloody noses, easy bruising or prolonged bleeding?				
9	Has your child ever had any abnormal blood tests?				
10	Has your child ever had any problems with breathing, asthma or coughing?				
11	Has your child ever had any problems that make you concerned about his/her heart?				
12	Has your child ever had any significant problems with his/her mouth or teeth?				
13	Has your child ever had any bowel problems, including any bowel accidents?				
14	Does your child have ongoing problems with diarrhea?				
15	Has your child ever had any ongoing episodes of vomiting?				
16	Has your child ever had any significant problems with appetite?				
17	Has your child ever had any significant problems with weight?				
18	Has your child ever had any significant problems with his/her muscles or joints?				
19	Has your child ever had any problems with swelling in any part of his or her body?				
20	Has your child ever had any problems with his/her urinary tract or problems when he/she urinates?				
21	Has your child ever had any problems with his/her breasts/nipples or private parts?				
22	Has your child ever had any problems with how thirsty he or she is?				
23	Has your child ever had any problems with his/her ears or hearing?				
24	Has your child ever had any eye or vision problems?				
25	Has your child ever had problems with severe or frequent headaches?				

		EVER		Past 2 months	
		No	Yes	No	Yes
26	Has your child ever had any significant problems with dizziness?				
27	Has your child ever had any seizures?				
28	Has your child ever had any problems with involuntary movements?				
29	Is your child chronically tired or fatigued?				
30	Has your child ever had cancer?				
31	Has your child ever had a tumor?				
32	Has your child ever had any unexplained masses or growths?				
33	Have you ever had any other concerns about your child's medical health? If so, please explain below.				
33	Has your child ever had any significant problems sleeping at night?				
34	Has your child ever had any significant problems being too sleepy during the day?				
35	Has your child ever had problems where he/she had to move around a lot, or had a hard time sleeping, or was upset because it seemed like he or she wasn't comfortable in his/her body?				
36	Has your child ever had any problems with rituals, being flexible, repetitive behaviors or language?				
37	Has your child ever had any problems with hyperactivity or impulsivity?				
38	Has your child ever had any significant problems with refusing to follow directions that he/she understands?				
39	Has your child ever had any significant problems with aggression, irritability or getting frustrated easily?				
40	Has your child ever had significant problems (more frequent/severe than other kids) with meltdowns or agitation?				
41	Has your child ever had any problems with staying motivated?				
42	Has your child ever had ever any problems with worries or sadness?				
43	Has your child ever had any problems with hurting him/herself on purpose or wanting to die?				
43	Has your child ever had any significant problems with mood swings?				
44	Has your child ever had any problems with believing things that aren't true or seeing/hearing things that aren't there?				
45	Has your child ever had any problems with seeming not to know where he/she was or what was really happening?				
46	Have you ever had any other concerns about your child's behavior? If so, please explain below.				

Please use the space below to explain any "yes" answers.

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## FOLLOW-UP MEDICAL & BEHAVIORAL HISTORY QUESTIONNAIRE

**Please tell us if your child has ever experienced any significant problems with any of the following conditions SINCE YOU LAST COMPLETED THIS QUESTIONNAIRE.**

		Past 3 months	
		No	Yes
1	Has your child had any fevers?		
2	Has your child had infections?		
3	Has your child had any allergic reactions?		
4	Has your child had any new or worse problems with autoimmune disorders?		
5	Has your child had any new rashes or abnormal skin lesions?		
6	Has your child developed jaundice (yellowing of the skin or eyes)?		
7	Has your child received a blood transfusion?		
8	Has your child had any new problems with bloody noses, easy bruising or prolonged bleeding?		
9	Has your child had any abnormal blood tests since their last visit?		
10	Has your child had any new or worsening problems with breathing, asthma or coughing?		
11	Has your child had any new or worsening problems with their heart?		
12	Has your child had any new problems with his/her mouth or teeth? (Do not include losing baby teeth.)		
13	Has your child had any new or worsening bowel problems, including bowel accidents?		
14	Has your child had any new or worsening diarrhea?		
15	Has your child had any new or worsening episodes of vomiting?		
16	Has your child had any new or worsening problems with appetite?		
17	Has your child had any new or worsening problems with weight?		
18	Has your child had any new or worsening problems with his/her muscles or joints?		
19	Has your child had any new or worsening swelling in any part of his or her body?		
20	Has your child had any new or worsening problems when he/she urinates?		
21	Has your child had any new or worsening problems with his/her breasts/nipples or private parts?		
22	Has your child had any changes in thirstiness?		
23	Has your child had any new or worsening problems with his/her ears or hearing?		
24	Has your child had any new or worsening eye or vision problems?		
25	Has your child had any new or worsening headaches?		
26	Has your child had any new or worsening problems with dizziness?		
27	Has your child had any new or worsening seizures?		
28	Has your child had any new or worsening problems with involuntary movements?		
29	Has your child had any new or worsening chronic tiredness or fatigue?		



## 15.0 APPENDIX 2: BIOLOGICAL SAMPLES

The following biological samples will be obtained and stored for future studies:

Subject	Sample Type	Collection Vessel	Amount
<b>Patient</b>	Blood sample for immune panel and DNA	Green top tube	2 x 3 mL
	Blood sample for iPSCs	Purple top tube	2 mL
	Blood sample for RNA	PAX gene tube	2.5 mL
	Urine sample	Cotton balls	2 mL (1 mL per tube)
	Saliva	Sponge	2 mL
	Buccal Swab	Kit	back up if unable to obtain blood
<b>Parents of Patient</b>	Blood samples from parents	Purple top tube	4 mL

## 16.0 APPENDIX 3: SEDATION GUIDELINES

### DukeACT Sedation Guidelines

Procedural sedation is intended to result in a depressed level of consciousness that allows the patient to maintain independent and continuous airway control. *All sedation will be performed by a pediatric anesthesiologist* per their practice guidelines and based on each child's clinical status. The guidelines for the sedation procedure are detailed below:

#### *Equipment:*

MRI compatible oxygen delivery system  
Oxygen saturation monitor and probe  
Appropriate sized ambu bag and mask  
Suction  
Blood pressure monitoring capability  
Continuous heart monitoring  
End tidal CO<sub>2</sub> monitoring device  
Supplies to obtain IV access

#### *Premedication: (for IV placement/sedation)*

Midazolam 0.5mg/kg orally or 0.2mg/kg intranasally, administered 15-30 minutes prior to the procedure. A healthcare professional will remain with the patient to monitor level of consciousness through direct observation.

#### *Sedation options:*

Dexmedetomidine (load of up to 2mcg/kg and an infusion of 0.2-2.0mcg/kg/hr) IV

Or

Propofol (load 0.5-1.0mg/kg) IV and continue as a continuous infusion until procedure is completed.

#### *Intra-Procedure:*

- a) Patients will be continually monitored by MD/RN for signs of hypoventilation, hypotension, apnea and bradycardia.
- b) Continuous monitoring of heart rate, oxygen saturation using pulse oximetry, and end tidal CO<sub>2</sub> using the MRI safe devices will be performed during the procedure. Blood pressure will be obtained at regular intervals.
- c) Patients will receive supplemental oxygen throughout the procedure.
- d) At no time will a patient be left unattended.

#### *Post-Procedure Monitoring:*

Continuous monitoring of heart rate and oxygen saturation using pulse oximetry will be performed until the patient is awake.

*Discharge:*

A. Patients may be discharged when the following criteria are met:

- a) A RASS (RICHMOND AGITATION SEDATION SCALE) Score of -1 or 0.
- b) Responsiveness and orientation reflects pre-procedure status
- c) Patient is able to ambulate with minimal assistance or mobility returned to baseline.

B. Patients will be discharged in the care of their caregiver with post-procedure instructions, including a contact phone number in case of emergency.